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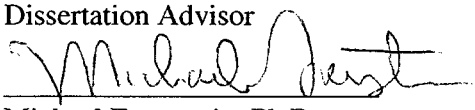

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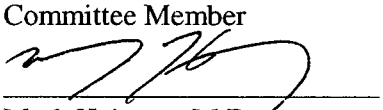

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# RELATIONSHIPS AMONG NON-INVASIVE PREDICTORS OF SUDDEN CARDIAC DEATH DURING MENTAL STRESS AND EXERCISE IN CORONARY ARTERY DISEASE PATIENTS WITH KNOWN ARRHYTHMIC VULNERABILITY

by

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**Abstract**

Sudden cardiac death (SCD) claims 350,000 lives each year. In 80% of cases of SCD, malignant arrhythmias are the main cause. Primary prevention of SCD is often an invasive and costly procedure involving implantation of cardioverter-defibrillators (ICD). Moreover, it has been challenging to identify individuals at higher risk of SCD who may benefit from ICD prophylaxis. There is an urgent need for accurate indices to identify individuals at risk for malignant arrhythmias contributing to SCD.

Several non-invasive markers have been proposed as risk stratifiers for SCD; however, none of these markers has proven to be sufficient as independent predictors of future malignant arrhythmias. It has been suggested that a complex interaction of electrical instability of the heart, compromised autonomic regulation of the heart, and damaged myocardial substrate, collectively may lead to SCD. Given that mental stress occurs frequently during daily life, there is also a need to understand how these markers interact during mental stress.

The current study determines the relationship among non-invasive markers of myocardial vulnerability (QT variability and inducible ischemia), autonomic activity (high frequency and heart rate recovery), and myocardial substrate (ejection fraction) to T-wave alternans, used as a proxy for predisposition to sudden cardiac death, in patients with known arrhythmic vulnerability. These relationships were examined under two acute laboratory challenges (exercise and mental stress) known to elicit different cardiac and peripheral hemodynamic patterns in healthy individuals and patients with cardiac disease.

The results of the study revealed moderate relationships between TWA and a marker of compromised myocardial substrate (EF) $r = -0.43$ and $r = -0.38$, and between

TWA and a marker of myocardial vulnerability (QTVI) r =0.48 and r =0.52, during mental stress and exercise, respectively. The present study did not find relationships among markers of autonomic vulnerability (HF and HRR), and TWA. The strength and direction of the relationships between non-invasive markers of malignant cardiac arrhythmias and TWA did not differ between exercise and mental stress, $Z = 1.04$, $p = 0.30$. Individual associations of these markers to TWA did not statistically differ from additive effects of these markers on TWA.

These findings suggest that resting QTVI and EF reflect similar phenomena as TWA – increased arrhythmic vulnerability. The moderate correlation of QTVI and EF with TWA implies that these indices are not redundant and may have unique contribution in the SCD risk stratification of patients in addition to TWA. The lack of differences in the associations of the markers between exercise and mental stress is consistent with the notion that both stressors are similarly pro-arrhythmic. While the relationships between markers remained similar between mental stress and exercise the performance of the individual markers differed across the stressors, which is consistent with different underlining physiology of the two challenges. Finally, the lack of relationships among markers of autonomic vulnerability and markers of pro-arrhythmic vulnerability, may suggest that dysregulation of autonomic system does not measure the same physiological phenomena as TWA- increased risk for arrhythmic events.

## Glossary of Terms

BRS—Baroreflex sensitivity

CHD—Coronary heart disease

CVD—Cardiovascular disease

EF—Ejection fraction

EPS—Electrophysiology study

HF—High frequency (component of heart rate variability)

HR—Heart rate

HRR—Heart rate recovery

HRV—Heart rate variability

ICD—Implantable cardioverter defibrillators

MI—Myocardial infarction

MMA—Modifying moving average (TWA measure)

MTWA—Microvolt T-wave alternans

QTVI—QT variability index

PVS—Programmed ventricular stimulation

SCD—Sudden cardiac death

SPECT—Single photon emission computed tomography

SVR—Systemic vascular resistance

TWA—T-wave alternans

VF—Ventricular fibrillation

VT—Ventricular tachycardia

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## Introduction

Sudden cardiac death (SCD) accounts for nearly 50% of all cardiovascular deaths in the USA ("[ACC/AHA/ESC 2006 guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death--executive summary]," 2007; Zipes, et al., 2006). Death is often the first and only observed symptom of cardiac disease in patients, and therefore primary prevention of SCD is of great importance. SCD occurs predominantly due to ventricular fibrillation (VF, non-rhythmic and mechanically ineffective ventricular contractions) or ventricular tachycardia (VT, rapid ventricular contractions) (Zheng, Croft, Giles, & Mensah, 2001). The use of implantable cardioverter defibrillators (ICD) has been extremely effective in terminating malignant VT and VF (Narayan, 2006; Narayan, Smith, & Cain, 2005); however the identification of the individuals in need of ICD therapy remains challenging. There is an urgent need for accurate indices to identify individuals at risk for malignant arrhythmias contributing to SCD.

Leclercq and Coumel (1986), and later on Zareba and Moss (2003) proposed a "lethal triad" that, through complex interaction, collectively lead to SCD: electrical instability of the myocardium, compromised autonomic regulation of the heart, and damaged myocardial substrate (Leclercq, et al., 1986; Zareba & Moss, 2003). Various non-invasive markers are proposed to assess the three components of the lethal triad: myocardial vulnerability, autonomic nervous system dysfunction and damaged myocardial substrate (see figure 1). The present research will examine relationships among these components.

Two decades of research have linked T wave alternans (TWA), a marker of cardiac electrical instability, and reduced ejection fraction (a marker of abnormal substrate) to malignant arrhythmias, the leading cause of SCD (Shusterman, Goldberg, & London, 2006). Physiologic and mental stress lead to an increase of sympathetic and a decrease of parasympathetic influences on the heart (Frankenhaeuser, 1978; Krantz, et al., 1999; Lampert, Jain, Burg, Batsford, & McPherson, 2000; Thayer & Sollers, 2000). Autonomic changes during mental stress increase the likelihood of malignant arrhythmias. Patients with severe structural heart disease are at increased risk for spontaneous and inducible ventricular arrhythmias during mental stress (Kop, et al., 2004; Lampert, et al., 2000; Lampert, et al., 2002). However, the interrelationships among non-invasive markers assessing repolarization instability, autonomic dysregulation, and abnormal substrate during mental stress are not well understood.

The objective of this study is to determine the individual and combined predictive value of non-invasive markers of myocardial vulnerability, imbalances in the autonomic regulation of the heart, and myocardial substrate damage to TWA in patients at risk of experiencing malignant arrhythmias. Differences in the magnitude and direction of these relationships during exercise and during acute mental stress will be examined.

This proposal will describe the public health burden of SCD and primary physiological mechanisms causing SCD. The literature on non-invasive markers representing the three parts of the lethal triad will be reviewed, and the pathophysiological effects of acute mental stress on CVD will be described. The specific aims and hypotheses for the proposed study will also be delineated.

**Sudden Cardiac Death**

Despite improvements in the diagnosis and management of cardiovascular disease (CVD), CVD is still a leading cause of death in the United States (Thom, et al., 2006). In 2002, about 58% of adult deaths were attributed to CVD ("[ACC/AHA/ESC 2006 guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death--executive summary]," 2007; Zipes, et al., 2006). Coronary heart disease (CHD) accounts for 53% of deaths from CVD ("[ACC/AHA/ESC 2006 guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death--executive summary]," 2007; Zipes, et al., 2006), with sudden cardiac death (SCD) accounting for almost half of CHD deaths (C. S. Fox, Evans, Larson, Kannel, & Levy, 2004). Thus SCD accounts for over 15% of all adult deaths.

SCD is defined as unexpected death resulting from cardiac arrest (sudden stopping of the heart) within 1 hour of the onset of symptoms (Brugada & Andries, 1992; Rodriguez, et al., 1992). The most common symptoms of SCD are chest pain and shortness of breath. Unfortunately, cardiac arrest is often the only observed symptom of SCD (Fox, et al., 2004; Kannel, Wilson, D'Agostino, & Cobb, 1998; D. Muller, Agrawal, & Arntz, 2006). Over 85% of individuals with SCD die before reaching the hospital. Of those admitted for medical care, over half die before discharge. The recurrence rate for those who are discharged is very high. Such poor prognosis after an initial episode of SCD limits the effectiveness of tertiary prevention and highlights the importance of secondary prevention efforts for SCD.

**The Lethal Triad**

Ventricular tachycardia (VT) followed by ventricular fibrillation (VF), is the leading cause of SCD (Brugada & Andries, 1992; Brugada, et al., 1991; D. Muller, et al., 2006). Research also shows that VT, manifested by a high ventricular heart rate (HR>100bpm), is frequent in patients with ischemic heart disease (IHD). Post-MI patients are at the highest risk for SCD (Moss, Daubert, & Zareba, 2002; Wilber, et al., 2004), especially in the first 6 to 24 months after infarction (Brugada & Andries, 1992; Brugada, et al., 1991; D. Muller, Agrawal, & Arntz, 2006), and SCD occurs most frequently in those with an extensive infarction associated with severely reduced ejection fraction.

Early delivery of defibrillation is critical, and usually sufficient, to disrupt the malignant arrhythmia and save the patient's life (Cummins, 1993). Accordingly, the current standard of care for SCD prevention is the use of implantable cardioverter-defibrillators (ICD) in patients who are deemed to be at elevated risk for SCD (Goldberger & Lampert, 2006). In patients with prior life-threatening arrhythmias, several prospective, randomized controlled studies have demonstrated that ICD therapy is superior to other anti-arrhythmic therapies, including anti-arrhythmic drug therapy, in prolonging survival (Botto, et al., 2005). However, the increased use of ICDs resulting from randomized control trials (RCT) in highly refined populations may have only a modest impact on SCD incidents as the majority of SCD occurs unexpectedly, and SCD is often the only observed symptom of CHD (Fox, et al., 2004; Kannel, et al., 1998; D. Muller, et al., 2006).

Paradoxically, those subjects with extensive substrate abnormalities (i.e. severely reduced ejection fraction) who receive an ICD only experience an appropriate therapy, i.e. termination of arrhythmia with electroshock, in 30% at two years follow-up, suggesting that two-thirds of these individuals did not benefit from their implanted device. Present selection criteria lack sensitivity (too many false-negatives) and specificity (too many false-positives). Therefore, it is necessary to examine other markers of SCD risk in order to develop a better algorithm for risk-stratification of individuals for whom ICD therapy is considered beneficial.

Dysregulation of the autonomic control of the heart, including decreased vagal activity and increased sympathetic tone, has been linked to an increased susceptibility to lethal arrhythmias (Bruce, et al., 1977; Brugada, et al., 1991; Brugada, Talajic, Smeets, Mulleneers, & Wellens, 1989). Imbalances in autonomic regulation of the heart are unlikely to cause SCD without additional factors that increase the vulnerability of the myocardium. Zareba and Moss (2003) proposed the "lethal triad" to explain the difficulties in predicting SCD: in their formulation, SCD results from the complex interplay of myocardial vulnerability, dysregulation of autonomic control of the heart, and the presence of abnormal myocardial substrate (See figure 1). Myocardial vulnerability may be manifested through the presence of ischemia in the heart, QT variability (beat to beat fluctuation in QT interval on the ECG), spontaneous but nonsustained ventricular arrhythmias, and T wave alternans (changes in amplitude, width and/or shape of T waveform on the ECG). Dysregulation of the autonomic control of the heart may be measured by heart rare variability, a measure of variations in the HR, and baroreflex sensitivity, a measure of the homeostatic mechanism for maintaining blood

pressure. Myocardial substrate condition can be evaluated by examining wall motion abnormalities (inadequate in ward movement of ventricular walls during systole), reduced ejection fraction (a fraction of end-diastolic volume expelled from the ventricle), atrial fibrillation, prolonged QRS, and late potentials. According to Zareba and Moss (2003), these markers may be important precipitates of lethal arrhythmias and SCD.

***Myocardial vulnerability.*** Myocardial vulnerability is the predisposition of the myocardium to cardiac arrhythmias or "electrical" instability (Zareba & Bayes de Luna, 2005). In approximately 85% of SCD cases ventricular tachycardia or fibrillation is the initiating event. Furthermore, myocardial ischemia may trigger VT or VF. Therefore, the presence of ischemia or ventricular tachycardia during stress testing provides additional information on the susceptibility of the myocardium to electrical instability (Verrier & Nearing, 1994). T wave alternans (TWA) and QT wave variability are non-invasive markers of myocardial vulnerability (Zareba & Moss, 2003). In order to understand the processes leading to malignant arrhythmias it is important to review the physiology relevant to the cardiac cycle, ECG, and action potentials.

*Cardiac cycle.* The cardiac cycle, one heart beat, consists of two periods, diastole and systole. Diastole is a period of relaxation, during which the ventricles fill with blood and cardiac muscle is oxygenated. Systole is a period of contraction, during which the ventricles contract and force the blood to the lungs and through the systemic circulation. In this manuscript systole and diastole will refer to ventricular events unless otherwise specified.

The normal cardiac cycle involves several steps: (1) a generation of an action potential in the sinus node (SA) or the pacemaker; (2) a conduction of the action potential through the atria; (3) a delay of conduction in the atrioventricular node (AV); (4) a rapid conduction to the ventricles through the bundle of His (left and right branches); and (5) a conduction through the Purkinje fiber. See figure 2. Conduction of the action potential through the ventricles causes contraction of the heart muscle. Ventricular recovery occurs in a stereotypically geometrical pattern similar to the activation. In a normal heart, the AV node conducts impulses from the atrium to the ventricles, termed antegrade conduction. See figure 4.

The action potential is created through the interaction of ionic permeabilities of the cells and transmembrane ionic currents. In muscle tissue and Purkinje fibers, sodium ions ($Na^{+}$) rapidly enter the cell causing membrane depolarization. This rise in membrane voltage activates calcium channels, leading to influx of calcium ($Ca^{2+}$), and subsequent opening of intracellular calcium channels in the membrane of the sarcoplasmic reticulum membrane (SR). This increase in the concentration of the intracellular calcium level causes contraction through formation of actin-myosin cross-bridges. This phase of the action potential corresponds to a refractory period, during which further impulses cannot be conducted. The efflux of potassium ions ($K^{+}$) through activated $K^{+}$ channels restores the resting membrane potential (repolarization) and the removal of $Ca^{2+}$ from the cell deactivates the protein-cross-bridges, thereby relaxing cardiac muscle.

It must be noted that both the contraction and relaxation of cardiac muscle require adenosine triphosphate (ATP, which plays a central role in energy metabolism) and that

ATP formation requires oxygen. Inadequate delivery of oxygenated blood results in ATP depletion with seconds and is known as "ischemia." Ischemic depletion of ATP results in rapid derangement of ionic balances with a rise in intracellular $Ca^{2+}$ and $Na^{+}$ and extracellular $K^{+}$. This shift in ion concentrations depolarizes the cell, impairs cardiac relaxation and contraction, and facilitates pro-arrhythmic activity.

*Action potential effect on ECG.* The electrical activation of the atria is reflected by the P wave. The duration of the PR interval represents the time of conduction from the SA node to the ventricles. Electrical activation of the ventricles generates the QRS complex. The ST wave reflects ventricular recovery. See figure 3. Prolonged ventricular action potentials are reflected in the ECG through QT interval prolongation.

*The action potentials in the disease state.* In the normal heart, the AV node is the only pathway for conducting impulses from the atrium to the ventricles. If the heart allows conduction of an electrical impulse through an additional pathway, it can predispose a person to arrhythmia. The self-regenerating conduction of impulses, independent of the SA nodal rate, is called the re-entry phenomenon, and is the most common mechanisms of all arrhythmias, including ventricular fibrillation. There are 3 classical prerequisites for the development of the re-entry. (1) There has to be a difference in the refractory periods of cardiac muscle. This allows for conduction of the retrograde impulse in one area and the inhibition of conduction in the refractory area. (2) There has to be slow conduction in one of the areas of the cardiac muscle. One pathway has to conduct slowly enough that the excitability can be regained by another pathway

before the depolarization can make a complete cycle. (3) There has to be a unidirectional block in one of the pathways, possibly due to scarring or ischemia but sometimes due simply to the anatomic structure of the tissue. This unidirectional block will impair conduction in one direction and allow conduction of the impulse in opposite direction. See figure 4. Thus, re-entry occurs when an electrical impulse travels in a circle within the heart, instead of moving along a single main axis and terminating at the completion of a single wave of depolarization.

Previous myocardial infarction or presence of ischemic regions may manifest heterogeneous repolarization, slow conduction, unidirectional block, and be a source for premature ventricular beats that can initiate reentry. Ischemia may result in ATP depletion, subsequent failure of Na/K ATP-ase pump, and accumulation of extracellular potassium. The resultant change in the equilibrium of potassium depolarizes the cell, resulting in a decrease in excitable Na+ channels and depression of $Na^+$ current into the cell. This leads to slowed conduction of action potentials in the ischemic area, and heterogeneity of refractoriness. These circumstances greatly increase the likelihood of re-entrant conduction. The presence of post-infarction fibrosis (“scar”) interferes with normal cell-to-cell coupling, reducing the efficiency of electrical conduction and promoting action potential variability and slow conduction. Finally, the electrophysiology of damaged cells may be transiently altered by autonomic stimulation. Activation of the sympathetic nervous system causes an increase in calcium loading of the ventricle as well as in SA nodal automaticity, increasing heart rate. All of the above can result in transient increases in arrhythmia risk due to effects on triggered depolarizations and labile changes in action potential duration and morphology.

*Summary.* Cardiac cells are able to transmit impulses in multiple directions provided they are stimulated sufficiently during an excitable period. During the normal cardiac cycle, electrical excitation starts in the sinus node, moves to the atria, crosses the antrioventricular node, activates the bundles of His and finally reaches the ventricles. Recovery also has a stereotypically geometric pattern. Diseased states of the heart (e.g. ischemia) may lead to imbalances in the ions responsible for and recovery of the myocardium and this may create slowed conduction in some areas of the myocardium as well as loss of uniformity of repolarization and increased frequency of spontaneous depolarizations. Due to heterogeneous refractoriness, premature, slowly conducted impulses may stimulate tissue that is partially excitable while blocking in adjacent areas, allowing a curving wave of depolarization that can "re-enter" and result in a self-perpetuating arrhythmia (Lozano, Mandel, Hayakawa, Shine, & Eber, 1973; Moe & Mendez, 1966; Moe, Rheinboldt, & Abildskov, 1964; Watanabe & Dreifus, 1965).

*T-wave Alternans.* T-wave alternans was first described by Mines in 1913. The T-wave of the electrocardiogram (ECG) represents the repolarization (recovery of excitability) of the ventricular myocardium during diastole (or rest). It is believed that the susceptibility of the ventricles to fibrillation is related to the degree of heterogeneity of the repolarization (Adam, et al., 1984). A particular pattern of variations in the beat-to-beat T-wave morphology has been shown to be related to decreases in the ventricular fibrillation threshold (Adam, et al., 1984). This pattern usually cannot be visually

detected from the ECG, however it can be quantified in terms of a T-wave alternans index (Adam, et al., 1984).

The duration of the action potential is modified by many factors, particularly stimulation frequency. As heart rate increases, the action potentials throughout the ventricular wall shorten uniformly due to efficient cell-to-cell coupling. At high heart rates, even normal cardiac cells demonstrate repolarization alternans, where successive action potentials alternate between long or short durations (Krogh-Madsen & Christini, 2007). In cardiac muscle, where cells act as a pseudo-syncitium, there are two main patterns of repolarization: spatially concordant and spatially discordant (Krogh-Madsen & Christini, 2007). During spatially concordant alternans, the entire tissue exhibits the same type of action potential, and this phenomenon is observed in healthy hearts that have been pushed to high heart rates. During discordant repolarization, at least one region is out of phase and exhibits a different type of the action potential than the rest of the tissue, and this phenomenon is more frequently seen in structurally abnormal hearts where cell-to-cell electrical coupling has been altered due to intracellular scar or "slippage" from heart failure. Discordant alternans creates a repolarization gradient or non-uniformity of excitability and conduction velocity in cardiac muscle (Burton & Cobbe, 2001). This gradient in the repolarization of the heart muscle increases the likelihood of reentrant impulse propagation (Burton & Cobbe, 2001). Research shows that T wave alternans is inducible only during discordant repolarization (Pastore, Girouard, Laurita, Akar, & Rosenbaum, 1999). Thus, TWA may be an important predecessor of VF (Nearing & Verrier, 2002; Pastore, et al., 1999). The presence of ischemia may exacerbate the ionic and electrophysiologic instabilities in the

myocardium, thus escalating the level of TWA (Kovach, Nearing, & Verrier, 2001; Narayan, Smith, Lindsay, Cain, & Davila-Roman, 2006). This will further increase the vulnerability of the myocardium to an arrhythmic event (Burton & Cobbe, 2001; Moe & Abildskov, 1964; Moe, et al., 1964).

T-wave alternans measures. T-wave alternans (TWA) is the beat-to beat fluctuation in the magnitude of T waves that occurs every other beat (Shusterman, et al., 2006). See figure 5B. TWA can be measured through visual examination of ECG in rare circumstances (See figure 5A), but more typically through the use of spectral analytical techniques or time-domain analyses (Verrier & Nearing, 2003). Spectral analysis during controlled heart rate acceleration is the most widely applied method (Gehi, Stein, Metz, & Gomes, 2005). This is also called the microvolt T-wave alternans (MTWA) method. See figure 5C. MTWA is measured during exercise-induced elevated heart rate (HR) or atrial pacing, but it is of a little predictive value when measured at heart rates of greater than 115 beats per minute because even normal hearts will manifest TWA at high heart rates. The most widely used criteria for the spectral analyses of TWA are offered by the Cambridge Heart commercial system (Gehi, et al., 2005; Narayan, et al., 2006). Based on these criteria, positive TWA is defined as TWA sustained for > 1min; if HR is < 110 and less than 10% of abnormal beats, without alternans from breathing. Negative TWA is defined as the absence of positive TWA as long as a HR > 105 beats/min was achieved. The Cambridge Heart method or MTWA has very detailed criteria, requiring specialized proprietary equipment and an experienced cardiologist to interpret the results. The MTWA method also does not provide continuous data (Narayan, Smith, Lindsay, Cain, &

Davila-Roman, 2006). Due to the need to achieve target heart rate, TWA testing yields 20-40% indeterminate results, as cardiac patients may not be able to raise, achieve, and maintain the required heart rate (Tapanainen, Still, Airaksinen, & Huikuri, 2001; Verrier, et al., 2003).

In order to overcome these challenges and enable data collection in ambulatory subjects, a time domain method was developed (Verrier & Nearing, 2003; Verrier, Nearing, & Stone, 2002). This newer technique to measure TWA is called the Modified Moving Average (MMA). See figure 5D. The full technical details of MMA method are described in Nearing and Verrier (2002). Briefly, the ECG recordings are analyzed by dividing normal beats into odd and even groups, and morphology of the beats in each group is averaged over a few beats successively to create a moving average complex (Verrier & Nearing, 2003; Verrier, et al., 2002). TWA is computed as the maximum differences between the amplitudes of the odd-beat and even beat averages. See figure 5D. TWA measured as $\geq$ 15 seconds stratifies SCD risk for ambulatory ECG. MMA does not require the control of HR, and, in theory, is minimally disrupted by noise and other artifacts (Verrier & Nearing, 2003; Verrier, et al., 2002). The MMA method produces continuous values for the TWA. It does not require a detailed protocol, e.g. achieving target heart rate, and it can be analyzed from previously-acquired digitized ECGs, including ambulatory recordings (Cox, et al., 2007). MMA also measures shorter duration of TWA, 15 seconds versus 1 minute, than spectral analyses method.

With all the above listed potential advantages, MMA is a new algorithm, and requires further validation. There are only two studies which compared commonly-used and well-validated microvolt T-wave assessment to MMA T-wave analysis. These

studies demonstrated that in comparison to MTWA moving average analyses of T-wave overestimates the magnitude of T-wave alternans especially in presence of the noise on ECG recordings. A clinical trial that compared MMA and spectral analyses method (similar to the Cambridge heart algorithm) in 41 CAD patients during simultaneous ventricular and atrial pacing at 109 beats per minute (Cox, et al., 2007) indicated higher levels of TWA using MMA, as compared to the spectral analyses method, in predicting cardiac events (Cox, et al., 2007). This study retrospectively provided a value of TWA measured by MMA corresponding to "positive" TWA of MTWA. The cut-point for the MMA method to be identified as "positive" TWA is a value of TWA $\geq$ 10.75 microvolt versus 1.9 for the spectral method (Cox, et al., 2007). Interestingly, the investigators found that combining the two methods provided the best predictive model. Selvaraj and Chauhan (2009) compared the accuracy of both methods in ambulatory and synthetic ECG recordings. Investigators concluded that in presence of noise MMA falsely detects and overestimates T-wave alternans in comparison to microvolt T-wave assessment (Selvaraj & Chauhan, 2009).

Another important fact in T-wave alternans assessment is that since the magnitude of TWA is defined at 0.5 cycles/beat for MTWA and MMA methods, it measures the variability of repolarization processes represented only at higher frequency levels, and does not provide information on repolarization variability at lower frequency levels (Shusterman, et al., 2006). However, there is an increase in repolarization variability in other T-wave frequencies prior to arrhythmias (Shusterman, et al., 2006). This suggests that T-wave alternans analysis may not provide full picture of the electrical instability in the myocardium.

Predictive value of MTWA (Cambridge Heat Method) for SCD. Decades of research have linked TWA with ventricular arrhythmias and the basic mechanisms leading to their initiation (Narayan, 2006; Pastore, et al., 1999). This research shows that discordant alternans accompanies a state of electrical instability in the heart (Pastore, et al., 1999), because VF was always preceded by discordant alternans. The major cause of sudden cardiac death is VT or VF (Zheng, et al., 2001). Therefore, patients with inducible TWA may be at higher risk for SCD.

Rosenbaum et al (1994) first demonstrated the value of MTWA, using the Cambridge heart method, as a marker of arrhythmic risk. Twenty seven cardiac patients were followed for 20 months. Those who did not have significant levels of TWA had only a 6% VT event rate in comparison to 81% in patients exhibiting significant levels of TWA (Rosenbaum, et al., 1994). Alternans during repolarization was a significant and independent predictor for inducible arrhythmias on electrophysiologic testing: sensitivity, 81%; specificity, 84%; RR = 5.20 (Rosenbaum, et al., 1994). In addition, TWA performed as well as an electrophysiology study (EPS, which involves placing wire electrodes within the heart to induce and study tachycardic arrhythmias) in predicting spontaneous clinical ventricular arrhythmias (El-Sherif, Turitto, Pedalino, & Robotis, 2001; Rosenbaum, et al., 1994). A drawback of this study was the inherently invasive nature of right atrial pacing used to increase heart rate in order to elicit TWA. In the next series of studies, the target heart rate was achieved via bicycle exercise testing. Hohnloser et al., (1997; 1998) compared various markers, including left ventricular ejection fraction (LVEF, fraction of blood pumped by ventricle during a single beat), heart rate variability

(HRV), and QT dispersion, of arrhythmic risk in 95 patients undergoing ICD implantation. The end point of the study was the appropriate discharge of the ICD as documented by interrogation of the device (El-Sherif, et al., 2001). The results of multivariate Cox regression analysis demonstrated that TWA was the only significant independent risk factor for the appropriateness of the ICD therapy (Hohnloser, et al., 1998). Kaplan-Meier survival analysis showed that TWA ($p < 0.006$) and LVEF ($p < 0.04$) were the only significant univariate predictors. Surprisingly, EPS testing did not approach significance ($p < 0.2$). In a prospective multicenter study of 313 patients in sinus rhythm undergoing EPS, ventricular arrhythmia risk was also accessed using TWA during bicycle exercise and signal-averaged electrocardiography (SAECG) (Armoundas, Tomaselli, & Esperer, 2002; El-Sherif, et al., 2001). The end points were SCD, cardiac arrest, appropriate ICD therapy, and sustained VT (Gold, et al., 2000). Analysis revealed that TWA and EPS were the only independent predictors of the primary end points (Gold, et al., 2000). Thus, TWA appears to perform as well as EPS, and better than SAECG in risk stratifying patients for life threatening VT (Gold, et al., 2000).

Gehi et al. (2005) conducted a meta-analysis in order to determine the predictive value of microvolt T-wave alternans (MTWA). The data was retrieved from 19 studies published between years 1990 to 2004. Data from 2608 patients from various populations was analyzed, and the positive predictive value of MTWA for arrhythmic events was 19.3% (CI =17.7% to 21.0%; $p = .05$). Bailey et al. (2001), in their meta-analyses of arrhythmic risk stratification in post MI patients, had found that predictive value of patient's EF, ambulatory ECG, heart rate variability, signal-averaged ECG, and electrophysiologic study ranges from 13.3-26.8 %. This result indicates that the MTWA

method in risk stratifying the patients for ICD prophylaxes was comparable or superior to other methods including EF, ambulatory ECG, heart rate variability, signal-averaged ECG, and electrophysiologic study (Bailey, Berson, Handelsman, & Hodges, 2001). However, the study in (Gehi, Stein, Metz, & Gomes, 2005) also showed that the positive predictive value was dependent upon the population of patients studied. In particular, patients with congestive heart failure and positive TWA testing had higher relative risk of arrhythmic events during follow-up in comparison to post-MI patients with positive TWA testing.

Interestingly, some studies indicated that “indeterminate” TWA predicted death or sustained VT at least as well as “positive” TWA in CVD patients (Bigger & Bloomfield, 2007). Seven hundred and sixty eight patients with ischemic cardiomyopathy were tested for TWA and QRS duration (Chow, Kereiakes, et al., 2007; Chow, Saghir, et al., 2007). Sixty seven percent had positive or indeterminate (non-negative) TWA. After multivariate adjustment, non-negative TWA was associated with higher risks for mortality and appropriate ICD shocks (HR = 2.42; CI = 1.07 to 5.41; p = .04) in comparison to patients with negative TWA results. In contrast, QRS duration was NOT associated with ICD shocks or with mortality. Kaufman et al. (2006) specifically designed a study to test the hypothesis that an "indeterminate" microvolt T-wave alternans (MTWA) test has prognostic significance similar to a positive MTWA test. Five hundred and forty nine patients with left ventricular dysfunction participated in the study. During the 2 year follow up, 40 patients died, and 11 patients had non-fatal sustained ventricular tachycardia (SVA) (Kaufman, et al., 2006). The 2-year rate for death or SVA was 17.8% in patients with an "indeterminate" MTWA test compared to 12.3% in those

with a positive test. Furthermore, Chow et al (2007) showed that patients with negative TWA tests did not benefit from ICD prophylaxes and preserved left ventricular function, however patients with indeterminate TWA tests did (Chow, Kereiakes, et al., 2007; Chow, Saghir, et al., 2007; Kaufman, et al., 2006).

*Predictive value of MMA for SCD.* The MMA method of measuring TWA is fairly new, therefore the research in this area is limited. The first study assessing TWA using MMA method in order to identify patients at increased risk for arrhythmic events was conducted by Verrier et al. (2003). Twenty four hour recordings of ambulatory ECG of 15 post-MI patients from the prospective ATRAMI study who had SCD due to documented VT and 29 matched controls were analyzed. Patients with high TWA had seven times higher odds of malignant arrhythmias in comparison to controls (Verrier, et al., 2003). Kop et al (2004) studied TWA responses measured during exercise and mental stress in 23 ICD patients and 17 controls. TWA responses increased with exercise and mental stress, and TWA responses were higher in ICD patients in comparison to controls. After adjustment for heart rate increases, exercise and mental stress provoked increased TWA for ICD patients ($p < 0.05$), but not for controls ($p > 0.2$) (Kop, et al., 2004). Cox et al (2007) studied ECG recordings of 41 patients with impaired EF. TWA was measured during combined, simultaneous atrial and ventricular pacing. MMA $\geq 10.75$ μV predicted death or appropriate ICD discharge at $p = 0.06$ (Cox, et al., 2007).

*Summary*. The majority of cases of SCD death are associated with electrical vulnerability of the myocardium. TWA is an inexpensive, safe and non-invasive method of stratifying the individuals at higher risk for malignant arrhythmias. TWA testing ranked among the best risk stratifiers in comparison to EF, ambulatory ECG, heart rate variability, signal-averaged ECG, and electrophysiologic study. Furthermore, patients with positive or indeterminate TWA results are at higher risk of SCD death, overall mortality or malignant arrhythmias. At least one study showed that patients with negative TWA results did not benefit from ICD prophylaxis (Chow, Kereiakes, et al., 2007; Chow, Saghir, et al., 2007). However, some evidence also shows that the predictive value of MTWA significantly varies depending upon the population studied and the length of the follow-up (Gehi, et al., 2005). In theory, this may be overcome by combining TWA risk assessment with other methods reflecting different aspects of the repolarization processes and alternative mechanisms of SCD, such as autonomic imbalance and morphological damage of the heart muscle. Use of other arrhythmic vulnerability markers which may reflect the dispersion of repolarization processes at different frequencies than the alternans frequency may also improve the risk stratification of patients for SCD. Additionally, more studies are needed in order to further evaluate the clinical diagnostic value of various T-wave alternans assessment algorithms and potential strength and weaknesses among them.

*QT variability.* The duration of the QRS complex of the ECG represents ventricular depolarization. The T wave represents ventricular repolarization. Thus, the QT interval of the ECG represents the time required for the completion of both

ventricular depolarization and repolarization. The action potentials are not of the same duration across the heart muscle (Batchvarov & Malik, 2000). Recovery time of the action potential duration (APD) gets progressively shorter in the areas of the myocardium which activate later. Therefore, under normal conditions, the difference in APD compensates for the difference in activation time by shortening the recovery time. This mechanism protects the heart from re-entry (Batchvarov & Malik, 2000). Certain conditions and situations may delay cardiac repolarization, represented as QT interval prolongation, (Lanjewar, Pathak, & Lokhandwala, 2004) and this delay creates a situation when different areas of the myocardium have different levels of excitability. This dispersion increases the likelihood of re-entry.

In addition, the repolarization process is heart rate dependent (Zareba & Bayes de Luna, 2005). The function of potassium and sodium channels during repolarization is influenced by the heart rate. Heart rate dependence of the repolarization process as measured by the QT interval was described by Bazett and Frederica in 1920. They described the QT-RR relationship and established a heart rate correction formula for QT. Dynamicity of the repolarization process may also be described by beat-to-beat changes in the duration of repolarization (Zareba & Bayes de Luna, 2005). T-wave alternans and QT variability index (QTVI) represent aspects of this repolarization variability. However, T-wave alternans assesses the repolarization variability only at the 0.5 cycles/beat frequency. QT variability provides additional information on temporal instability of repolarization at lower and higher frequency levels (Berger, et al., 1997; Shusterman, et al., 2006). Shusterman et al (2006) analyzed ambulatory ECG of 42 CAD patients with known arrhythmic vulnerability. Spectral analyses were conducted to derive TWA (.45 to

0.5 cycles/beat frequency) and QT interval variability (0.3-0.4 cycles/beat frequency). Both TWA and QT showed an increase before the onset of ventricular tachyarrhythmia (Shusterman, et al., 2006).

QT variability index (QTVI). In 1997 Berger developed a QT variability algorithm adjusted for heart rate variability, QTVI. Using a semi-automated, template-matching algorithm, Berger (1997) demonstrated that cardiomyopathy is associated with an increase in the beat-to-beat variability of the duration of the QT interval, reflecting increased variability of repolarization out of proportion to heart rate variability (Berger, et al., 1997). As described above, QT variability in normal subjects is driven to a large degree by heart rate variability. Therefore, the measure defined by Berger, the QT variability index, statistically controls for heart rate variability.

According to Berger et al (1997 and 2003), "heart rate time series is constructed from the sequence of RR intervals using short-term, high fidelity ECG recordings. The heart rate mean (HRm) and variance (HRv) and QT interval mean (QTm) and variance (QTv) are computed from the respective time series. A normalized QT variability index (QTVI) is then computed:

$QTVI = \log10 [(QTv/QTm^2) / (HRv/HRm^2)]$. "(Berger, 2003; Berger, et al., 1997)

Therefore, the QTVI is a log ratio between the QT interval and the heart rate variability. Both, QT interval and heart rate variability are normalized by the squared mean of their respective time series (Berger, et al., 1997; Bigger & Bloomfield, 2007).

It should be noted since QTVI is a HR-dependent parameter, QTVI collected during exercise is significantly higher than QTVI at rest in both healthy subjects and those with cardiomyopathy (Haigney, et al., 2009).

Predictive value of QTVI for SCD. Both T wave alternans and QTVI are markers of the lability of the repolarization process, which increases the electrical instability of the heart muscle. It is unclear which measure is superior in identifying the patients prone to malignant arrhythmias. Additionally, it is not clear if QTVI is an accurate predictor of SCD in patients who have low severity of cardiac disease. For example, Atiga et al., (1998) followed 95 cardiac patients for an average 23.7 months after an invasive electrophysiologic study (EPS). EPS, HRV, QTVI, T-wave alternans and EF were taken at the beginning of the follow up. Only elevated QTVI at baseline identified patients who developed SCD ($p = .004$; OR = 12.5). In another study, Hohnloser and Cohen (1999) found that TWA was a better predictor of ICD-treated VT and VF than QTVI. In a more resent study of 396 subjects with chronic, nonischemic congestive heart failure (CHF) with an ejection fraction (EF) between 35 and 40 underwent a 5-minute ECG recording to calculate the QTVN and QTVI. The participants were followed for 5 years. The multivariate survival model indicated that QTVI, but not QTVN, was a significant predictor of SCD and total mortality in that group of patients (Piccirillo, et al., 2007). Haigney and colleagues (2004) found a strong association between the QT variability index and the arrhythmic events, VT and VF, as documented by ICD interrogations in 817 MADIT II patients. The use of QT normalized for the square of the mean QT (QTVN), not adjusted for HRV, improved the prediction of arrhythmic events. In this

study, multivariate Cox regression analyses, controlling for race and time after MI, showed that top-quartile QTVN was independently associated with VF and VT (Haigney, et al., 2004). In MADIT II, even patients in the lowest quartile for QTVN still had a 17% incidence of VT and VF. These findings suggest that QT variability may not be sufficient as predictor to identify patients at low risk for malignant arrhythmias. Alternatively, the detection of VT and VF by ICD may be too "soft" as and "endpoint," since many VT events did not result in cardiac arrest or SCD. There is no study of QTVI or QTVN that uses the hard endpoint of total mortality in a lower risk group.

Summary. An increase in beat-to-beat changes in the repolarization process leads to increases in the electrical instability of the heart. This may increase the likelihood of re-entry arrhythmias. QT variability, similar to TWA, is one of the markers of electrical instability of heart muscle. It may provide additional information on the dispersion of the repolarization processes, especially at lower frequencies, which are not assessed by the TWA method. In contrast to TWA it can be assessed at rest. However, at present, based on findings of clinical trials, QTVI cannot be used as an independent predictor of future arrhythmic events, especially in patients with low severity of cardiac disease.

*Ischemia.* Myocardial ischemia is defined as insufficient blood flow to the muscle tissue of the heart. The most common symptom of an acute myocardial ischemia is pain in the chest region or angina pectoris. Myocardial infarction is a prolonged ischemic event, which results in the necrosis of myocardial tissue. Approximately 65% of cases of SCD are linked to ischemia (Podrid & Myerburg, 2005). The presence of acute ischemia

can lead to VT and VF. VT and VF are the most common sequences of events leading to SCD (Rubart & Zipes, 2005). Therefore, the presence of an inducible ischemia during exercise or mental stress testing may serve as another non-invasive marker of SCD risk.

Mechanism linking ischemia to arrhythmias. An increased likelihood of malignant arrhythmias during acute ischemia is due to the phenomenon of re-entry as described above (Bardou, et al., 1995; Rubart & Zipes, 2005). Reentry is promoted by significant changes in intra- and extracellular ionic concentrations in myocytes (Rubart & Zipes, 2005). The mechanisms of ionic interactions are not completely understood. However, it is understood that ischemia causes cellular $K^+$ loss and consequent extra cellular $K^+$ accumulation resulting in continuous membrane depolarization coupled with other factors. Conduction is slowed and, through re-entry, VT/VF may be promoted (Bardou, et al., 1995; Rubart & Zipes, 2005; Wu, Wu, Olgin, Miller, & Zipes, 2001; Wu & Zipes, 2001). Ischemia, or hypoxia at the cellular level, also causes an increase in cellular $Na^+$ ionic concentration, indicating that the $Na^+$/ $K^+$ ATPase is not functioning properly. This may be due to a decrease in ATP formation during ischemia (John, Kondo, Wang, Goldhaber, & Weiss, 1999). An increase in intracellular $Na^+$ triggers an increase in intracellular $Ca^{2+}$ concentration. This change also contributes to sustained depolarization and heterogeneous prolongation of the refractory period, which, in turn, creates conditions for the re-entry.

Measuring ischemia. Assessment of the ST segment depression of the ECG is the most widely used method to measure the presence of myocardial ischemia (Stern, 2002;

Strike & Steptoe, 2002). It is a non-invasive method of measuring myocardial ischemia and it is easy to administer. The ST segment of the ECG reflects ventricular recovery or the relaxation phase of the cardiac cycle. During this phase, the myocardium is supplied with oxygen. Ischemic conditions in the heart lead to prolonged depolarization, which, in turn, alters the recovery. Therefore, on the ECG, the depression of the ST segment reflects myocardial ischemia. Significant depression is indicated if the ST segment is at least one box below the baseline (approximately 1 mm), as measured at two boxes after the end of the QRS (Gibbons, et al., 2002).

It is important to note that, in asymptomatic (without chest pain) patients, ST segment depression during exercise testing yields a high number of false positive results (Berman, Rozanski, & Knoebel, 1987). For example, the likelihood of an asymptomatic 50-year-old man in the U.S. with positive exercise testing (ST-segment depression) having CVD is only 17%. Contrary to this statistic, in situations where the prevalence of the CVD is high based on symptom presentation (e.g. angina pectoris) the ST segment depression is much more likely to be a good indicator of CVD and will have a greater predictive value for myocardial ischemia (Berman, et al., 1987). Thus, in asymptomatic individuals, the presence of ST segment depression during exercise testing should not be labeled as silent myocardial ischemia.

Another measure of ischemia that has higher predictive value for detecting ischemia is regional left ventricular wall motion abnormalities (WMA) during stress (Rozanski, Krantz, & Bairey, 1991). Wall motion abnormalities are impaired inward movement of the ventricular wall during each contraction (i.e., at systole). WMAs tend to occur earlier in the ischemic process than ECG abnormalities. Therefore, WMAs serves

as an early mechanical marker of ischemia, which is easily detected by echocardiography or radionuclide assessments (Rozanski, et al., 1982; Rozanski, et al., 1991).
Changes in ejection fraction (EF, fraction of blood pumped out during each bit) from rest to stress can be used as another marker of ischemia. During exercise, a normal heart experiences an increase in EF, in comparison to resting EF. In an ischemic heart, there is no change or even a decrease in EF from rest to stress.

Gated single photon emission computed tomography (SPECT) is also used in order to identify perfusion deficits in the myocardium. SPECT is a tomographic imaging technique utilizing gamma rays, and provides 3D information on perfusion, represented by cross-sectional slices through the patient's heart. The underlying principle of this method is that under conditions of stress, diseased myocardium experiences less blood flow when compared to normal myocardium. A cardiac specific radiopharmaceutical (e.g., $^{99m}$Tc-tetrofosmin or $^{99m}$Tc-sestamibi) is administered. Following administration, the heart rate is elevated through exercise, mental stress or pharmacological substances to induce myocardial stress. Performing SPECT imaging following stress highlights the distribution of the radiopharmaceutical and, thus reveals the relative blood flow to the different areas of the myocardium. The isotope is also administered during the resting state and images of myocardial perfusion are taken. A diagnosis is determined by comparing stress images to a set of rest images. As the isotope redistributes slowly, it is not usually possible to perform both sets of images on the same day; therefore, a second visit is necessary 1-6 days later. However, if SPECT images are normal during stress, the resting images are not required. It has been demonstrated that gated SPECT has an overall accuracy of about 83%, sensitivity of 85%, and specificity of 72% in diagnosing

the myocardial perfusion deficits (Elhendy, et al., 2002). In addition, it has high reproducibility (Verberne, Dijkgraaf, Somsen, & van Eck-Smit, 2003; Yoshikai, et al., 1999), as well as high correlations with other measures of ischemia (e.g., ventriculography, echocardiography) (Choragudi, et al., 2001)

Value of ST-segment depression as a predictor of SCD. There are limited data indicating that the presence of silent ischemia or ischemia without chest pain, detected via Holter monitoring, may indicate an increased risk for sudden death (Zareba & Moss, 2003). Studies performed on post-MI patients in their pre-discharge period appear to indicate that the presence of ST segment depression detected via Holter monitoring during exercise may be predictive of adverse cardiac events, including SCD. For example, Rywik and colleagues (2002) performed treadmill exercise tests on 1,083 participants who were free of any clinical CVD. Seventy six subjects developed angina pectoris, coronary death, and MI over a mean follow up of 7.9 years. ST segment depression during exercise testing was an independent predictor of adverse coronary events, such as angina pectoris, MI, and coronary death, in this asymptomatic population ($RR = 2.73$, $p = .04$) (Rywik, et al., 2002). In another study of 708 survivors of initial MI (Bigi, Cortigiani, Gregori, De Chiara, & Fiorentini, 2004), ST-segment depression during exercise testing predicted cardiac death during the 32-month follow-up (Bigi, et al., 2004).

Despite these findings, results from other studies bring into question the reliability of ST-segment depression as a marker of future adverse cardiac events. For example, 163 patients with acute MIs underwent exercise testing and 24-hour ambulatory

electrocardiographic monitoring (Henry, Kennedy, & Crawford, 1987). During the 2-year follow-up, 10% of patients died, 9% had a recurrent MI, and 28% underwent artery bypass surgery. Electrocardiographic ST-segment depression during 24-hour ambulatory monitoring was not a significant predictor of future adverse cardiac events in this population (Henry, et al., 1987). Okin et al (1991) followed 3168 asymptomatic (without known cardiac disease) men and women who underwent exercise testing for an average of 4.3 years. During the follow-up, there were 65 adverse cardiac events (4 SCD cases, 24 MIs and 37 incidents of angina pectoris) (Okin, Bergman, & Kligfield, 1991). A Cox proportional hazard model with adjustment for age and sex was used to analyze the results. Positive exercise electrocardiogram (ST-segment depression) was not predictive of reported adverse events (Okin, et al., 1991).

Value of gated SPECT as a predictor of SCD. The majority of the studies linking ischemia measured by SPECT to arrhythmic events are done in patients who are already diagnosed with CVD. Paganelli et al. (2001) studied 90 MI survivors. Patients were divided into two groups: those with inducible arrhythmias (n = 24) and those without inducible ventricular arrhythmias (n = 66). Patients were comparable in terms of EF, location and the size of the infarct, and extent of the coronary disease. Forty-two percent of patients in group 1 had myocardial ischemia measured by SPECT in comparison to 25.7% of patients in group 2. The differences between groups regarding the presence of ischemia were statistically significant at $p < .05$ (Paganelli, et al., 2001). These results, although based on small numbers of subjects, may indicate that in post MI patients the presence of ischemia measured by SPECT may be relevant to an increased arrhythmic

risk. Isoda et al (1999) evaluated 88 patients with acute MI who underwent dual SPECT. During the follow-up period of 7 years, 29 (33%) patients had a cardiac event: 1 cardiac death, 3 with recurrent MI, 17 with coronary revascularization, and 8 with recurrent angina. Patients who tested positive for myocardial ischemia had higher likelihood of adverse cardiac events compared to patients who did not exhibit ischemia (Isoda, et al., 1999).

Krause et al. (2005) investigated 47 post-MI patients. In this sample thirty three patients suffered from ventricular arrhythmias. All patients underwent (99m) Tc tetrofosmin SPECT testing. Patients with higher levels of perfusion deficit were at increased risk of arrhythmic events, even after adjusting for symptomatic chronic heart failure (CHF), aneurysms, and age (Krause et al., 2005). Furthermore, 94% of arrhythmic patients displayed perfusion deficits in comparison to 64% non-arrhythmic patients (Krause et al., 2005). Another study of patients with normal EF and without myocardial ischemia (SPECT) showed that ventricular complex arrhythmias during exercise testing were not associated with adverse cardiac events during 1.5 years of the monitoring period. None of the patients with ventricular complex arrhythmias during exercise had angina pectoris, SCD, or MI at follow-up (Camilletti, et al., 2004).

Summary. Ischemia may lead to VT and VF resulting in cardiac arrest. Therefore, patients with inducible ischemia may be at a greater risk of SCD. However, these associations were found only in patients with already known arrhythmic vulnerability. It is not clear if inducible ischemia is a sufficient predictor of SCD in patients who are free

of known CVD. Additionally is not clear if presence of myocardial ischemia during exercise-testing is associated with arrhythmias.

***Autonomic nervous system and SCD.*** Numerous experimental and observational studies have showed that changes in the autonomic regulation of the heart may increase the risk of malignant arrhythmias and sudden cardiac death in cardiac patients. In general, increased sympathetic modulation of the heart and decreased parasympathetic modulation decrease the threshold for ventricular fibrillation when animals or humans have an ischemic challenge (e.g. exercise or mental stress). Several markers have been proposed in order to identify the state of autonomic balance of the heart regulation. Indices such as heart rate variability (HRV), baroreflex sensitivity, heart rate turbulence and heart rate recovery allow researchers to study the state of parasympathetic and sympathetic modulation on the heart. This section reviews the physiology of the autonomic heart regulation and the application of HRV and heart rate recovery in identifying patients at the higher risk for SCD.

*Role of the autonomic nervous system.* The autonomic nervous system (ANS) helps to maintain homeostasis through control of arterial pressure, gastrointestinal secretion, and body temperature among other functions. Essentially all of the organs and systems of the body perform functions that help to maintain homeostasis for the organism in the environment (Guyton & Hall, 2000). This homeostasis is crucial for the survival of the organism (Selye, 1954b). The autonomic nervous system, which is activated by centers located in spinal cord, brain stem and hypothalamus, is one of the most powerful

systems in maintaining this constant state. The ANS includes the sympathetic and parasympathetic nervous systems. Sympathetic stimulation causes excitatory effects in some organs, such as arterioles and inhibitory effects in others, such as bladder. Parasympathetic stimulation also displays selective excitation and inhibition (Guyton & Hall, 2000). Most of the organs are dominantly controlled by one or another branch of the ANS. However, in the heart, bronchi, and liver the two systems act reciprocally.

The parasympathetic nervous system tends to be highly specific to an organ or system in its activation, while the sympathetic nervous system is capable of producing a mass discharge in almost all its components (McEwen, 2002; Selye, 1975a). This mass discharge is called an alarm reaction or stress response (Mason, Mangan, Brady, Conrad, & Rioch, 1961; Selye, 1975b). This frequently occurs when the hypothalamus is activated by psychological stress, like fright or anger, or by physical stress like injury (Cole, Foody, Blackstone, & Lauer, 2000; Mason, 1968a, 1968b). This mass discharge directly increases the ability of the body to perform vigorous muscle activity by enhancing the work of certain organs and systems and inhibiting others. For example, the discharge increases mental concentration, heart contractility, heart rate, blood pressure, and decreases the work of the organs that are not needed for motor activity (Guyton & Hall, 2000).

*Autonomic regulation of the heart.* The heart is supplied with both sympathetic and parasympathetic nerves. The parasympathetic nerves stem from the vagal nerve and are distributed to the sino-atrial (S-A) and atrio-ventricular (AV) nodes of the conduction system of the heart (Zipes, Barber, Takahashi, & Gilmour, 1983). There are also vagal

efferents to the atrial and ventricular myocardium, although these are significantly less numerous as compared to nodal tissue (Zipes, et al., 1983). Stimulation of the parasympathetic nerves to the heart causes the neurotransmitter, acetylcholine, to be released from nerve endings. Acetylcholine slows the transmission of the impulses to the A-V node, which decreases HR and slightly decreases the strength of the heart muscle contraction and the rate of heart pumping (Guyton & Hall, 2000). This, in turn, decreases cardiac output and cardiac demand for the oxygen. Under resting conditions the parasympathetic influence, vagal tone, dominates that of the sympathetic influence on the heart in healthy individuals (Bernardi, Saviolo, & Spodick, 1989).

The sympathetic nerves are distributed to all parts of the heart, with increased concentration in the ventricles. Stimulation of these sympathetic fibers has a direct effect on the heart, causing release of norepinephrine from the nerve endings. In addition, the overall activation of the sympathetic nervous system causes a discharge of epinephrine and norepinephrine into the blood stream from the adrenal medulla (Guyton & Hall, 2000). Norepinephrine and epinephrine increase the heart rate and the force of myocardial contraction (Bernardi, Saviolo, et al., 1989), which increases ejection fraction and the volume of blood pumped by the heart, resulting in an increased demand by the heart muscle for oxygen (Guyton & Hall, 2000).

Therefore, the parasympathetic nervous system produces direct inhibitory effects on the heart and the sympathetic nervous system produces direct, through nerve fibers, and indirect, trough blood stream, excitatory effects on the heart muscle. This sympathetic/parasympathetic control of the heart, maybe illustrated through baroreflex mechanism.

Baroreflex. In cardiovascular physiology, the baroreflex is one of the body's homeostatic mechanisms for maintaining blood pressure. It is a short-term regulator of the blood pressure utilizing ANS. Specialized neurons (or baroreceptors) in the aortic arch, carotid sinuses, and atria are sensitive to fluctuations in blood pressure. The baroreceptors are stretch-sensitive mechanoreceptors. The increase in blood pressure stimulates the receptors and this information is signaled to the vasomotor center of the brain stem. Activation of the neurons in the brain decreases the sympathetic stimulation on the heart and vessels and increases the parasympathetic effect. This results in a decrease of the systemic blood pressure. The decrease in blood pressure results in decreased stimulation of the receptors, and therefore, decreased output to the brain stem. This causes an increase in sympathetic stimulation and a decrease in parasymapathetic stimulation, resulting in the elevation of blood pressure.

Baroreflex provides a negative feedback loop in which an elevated blood pressure reflexively decreases blood pressure. Similarly, decreased blood pressure depresses the baroreflex, which then blood pressure.

Predictive value of baroreflex sensitivity for SCD. Baroreflex sensitivity (BRS) has been used in clinical practice as a measure of baroreflex in order to stratify mortality risk after myocardial infarction (MI). Farrell et al (1991) compared the predictive powers of heart rate variability (HRV, beat to beat variation in the heart rate) and BRS in identifying patients at elevated risk for arrhythmic events. Sixty eight patients underwent electrophysiological test 9-10 days after infarction. The patients with lower BRS had the

lowest threshold for arrhythmia induction, by programmed ventricular stimulation (PVS). BRS responses predicted arrhythmic vulnerability better than SDNN (standard deviation of normal intervals) (Farrell, et al., 1991). In another study, data on left ventricular ejection fraction (LVEF), 24-hour ECG recording, and BRS from 1071 patients after MI from the ATRAMI was analyzed (La Rovere et al., 2001). During an average 21 month follow up, 43 patients experienced cardiac death and 30 patients experienced SCD. Decreased HRV and BRS were independent predictors of mortality. Use of all 3 (EF, HRV and BRS) increased the significance of the association by 22 (La Rovere et al., 2001). According to Bigger et al (2007) these findings warrant a future research in order to see if they could be developed into a clinically significant tool for identifying a subgroup of patients at risk for SCD.

Resting heart rate. Another simple way of measuring the state of sympathovagal balance is resting HR (Soliman, Elsalam, & Li). At rest, the heart is under dominant control of the parasympathetic nervous system. In case of diminished parasympathetic tone and/or increased sympathetic tone the resting HR will be elevated (Lauer et al., 2009). Findings of several epidemiological studies have demonstrated that elevated resting HR is associated with increased risk for cardiovascular disease and cardiovascular mortality (K. Fox, et al., 2007; Palatini, 2009; Reil & Bohm, 2007). However, there are only a few studies which have investigated the relationship of resting HR to SCD and malignant arrhythmias (Soliman, et al.). Soliman and colleagues (2009) investigated 867 patients who underwent 24 hour Holter monitoring and were followed for 2 years. It was found that elevated resting HR was an independent predictor of arrhythmic events during

the follow-up ($p<0.05$). It is important to note that resting HR is impacted by cardiovascular medication (e.g., beta-blockers). Use of beta-blockers decreases the HR, thereby influencing the clinical picture of the state of sympathovagal balance. In short, more investigations are warranted in order to study the link between elevated resting HR and increased arrhythmic vulnerability, taking into account the beta-blocker use in study population.

Heart rate recovery as a marker of autonomic regulation. Heart rate is one of the simplest and least invasive markers of the autonomic regulation of the heart. At rest, the heart and resting heart rate (HR) are under dominant control of parasympathetic mechanisms. During mental challenge or exercise the acceleration in HR is driven by sympathetic mechanisms (Gibbons, Balady et al. 2002; Thayer and Lane 2007). Therefore, resting HR can be a rough indicator of a state of parasympathetic balance. An increase in heart rate is a determinant of myocardial oxygen demand and the rate of myocardial energy use (Brook & Julius, 2000).

The decrease in HR after termination of exercise has been termed HR recovery. The decrease in HR in the first minutes after exercise has been shown to be a marker of the inhibitory effect on the heart of vagal reactivation following the termination of the sympathetically activated state of exercise (Shetler, et al., 2001). A delayed heart rate recovery after exercise indicates an increased sympathetic tone or lack of physiological increase in vagal tone through the feed back loop (described in baroreflex) (Israel, 2007). Reduced heart rate recovery, has been linked to the development and worsening of CVD

(Thayer & Sollers, 2000). Rapid recovery is associated with better health and decreased risk of mortality (Lauer & Froelicher, 2002).

Measuring heart rate recovery (HRR). Heart rate recovery is one of the simplest and least invasive measures used in cardiovascular diagnostics (Thayer & Lane, 2007). Most investigators simply measure the change in HR from peak exercise to minute 1 or 2 of recovery or consider the slope of the HR decline. Even though the heart rate recovery measure seems very simple, there are several methodological issues that must be addressed. The major concerns relate to maximal exercise protocol, the time elapsed for measuring recovery heart rate, and to the cutoffs used in determining pathological and normal heart rate recovery levels. For example, most of the studies use a maximal exercise protocol in order to measure HRR (Israel, 2007). However, Pierpont et al (2000) found that recovery heart rate values vary by 16 bpm among individuals during first 3 minutes after exercise cessation and more moderate exercise protocol should be used (Pierpont, Stolpman, & Gornick, 2000). In some studies, recovery heart rate was measured at 2 minutes after termination of exercise while others claimed that recovery heart rate at 30 seconds after exercise is most predictive of future cardiac events (Imai, et al., 1994). Different cut-off values to determine pathological results have been used (Israel, 2007), including decreases of 12-18 bpm after 1 minute and decreases of 42 bpm after 2 minutes (Gibbons, 2002). In addition, application of this paradigm to mental stress testing may be further complicated by the fact that mental stress does not have clear beginning or end.

Predictive value of HRR for SCD. The study of abnormal heart rate recovery and its relation with the susceptibility to malignant arrhythmias is fairly new. One of the key studies has been done by Smith and colleagues (2005). Post MI dogs (n = 105) were subjected to treadmill sub-maximal exercise. The next day, myocardial ischemia was induced in all animals. VF occurred in 66 animals. The analyses showed that HRR was higher in arrhythmia resistant animals (n = 39) in comparison to animals who suffered VF (Smith, Kukielka, & Billman, 2005). In another study, Jouven et al (2005) evaluated the ability of heart rate recovery (HRR) to predict SCD. They followed 5713 healthy men for 23 years. Individuals with lower HRR had an almost 2 fold increase risk of SCD, however this association did not hold for non-sudden death.

Several studies report that reduced heart rate recovery is associated with increased mortality (Cole, Foody, Blackstone, & Lauer, 2000). Cole et al (2000) investigated 5234 healthy individuals. A heart rate recovery of < 42 bpm at 2 minutes after exercise was associated with 2.58 increased risk for mortality from all-causes during 12 year follow up, even after controlling for resting HR, resting blood pressure, smoking and other associated factors. Shetler at al (2001) examined heart rate recovery in 2193 men (42% were post MI patients). During the 7 year follow up individuals with reduced heart rate recovery (cut off 22 bpm at 2 minutes) had a 2.6 times greater risk of mortality.

Summary. Heart rate recovery is a simple non-invasive tool for evaluating the state of cardiac autonomic balance. In several studies reduced heart rate recovery has been linked to increased mortality. There is also research indicating that reduced HRR may be a predictor of future SCD. However, the measure itself needs further

development to determine the abnormal cut-offs and optimal time to measure the recovery heart rate.

Heart rate variability. The heart period, or time between successive normal beats, is based on two components: the intrinsic firing rate of the SA node of the heart, and the collective input of sympathetic and parasympathetic nervous systems (McMillan, 2002). At rest, the rhythm of the heart is primarily under the control of the vagus nerve, which inhibits heart rate and the force of contraction (Bernardi, Saviolo, et al., 1989). During inhalation, vagal nerve activity is impeded and the heart rate increases due to a drop in intrathoracic pressure and consequent fall in systolic blood pressure. The change in blood pressure is sensed by the baroreceptor of the carotid sinus (Appel, Berger et al. 1989; Bernardi, Keller et al. 1989). During exhalation, the pattern is reversed (Appel, Berger, Saul, Smith, & Cohen, 1989). Therefore, the intervals between normal successive beats are not equal, and represent rhythmic activation and inactivation of sympathetic and parasympathetic control of the heart. This rhythmic fluctuation of the heartbeat is called respiratory sinus arrhythmia (Bernardi, Keller, et al., 1989). The magnitude of the changes in heart rate in response to changes in blood pressure reflects the sensitivity of the baroreceptor and the integrity of the parasympathetic nervous system.

There are other sources of HRV including circadian changes, standing up, head tilt, exercise, and psychological stress. The greatest variation of the heart rate occurs with circadian changes, the difference of the HR between night and day, ("Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing

and Electrophysiology," 1996; Kleiger, Stein, & Bigger, 2005). These changes are mediated by complex neurohormonal mechanisms (Kleiger, et al., 2005). In general, after waking, markers of sympathetic activity (HR, BP, catecholamine level) have been shown to rise quickly as a consequence of vagal withdrawal (Furlan, et al., 1990). This corresponds to a decrease in HRV because of vagal withdrawal. Therefore, the rhythm of the heart is also affected by circadian patterns of sympatho-vagal balance. In addition, standing up or tilting the head upright corresponds to a decrease of HRV, because of vagal withdrawal and minimal sympathetic activation of heart control (Bloomfield, et al., 1997; Bloomfield, et al., 2001).

The process of sympathetic activation and reduced parasympathetic activation of cardiac control also corresponds to a decrease in HRV (Carney, et al., 2000; Kleiger, et al., 2005). Pagani et al. (1991) reported that psychological challenges enhance sympathetic activity in dogs, therefore decreasing HRV.

Measuring HRV. Techniques for measuring heart rate variability can be divided into two categories: frequency domain and time domain methods. Time domain measures are the simplest to perform. Using this method, the heart rate at any point in time or the intervals between successive normal complexes are determined (Malik & Camm, 1993). Simple time domain measures can be calculated using the mean period between normal beats (NN intervals), the mean HR, standard deviations of the NN intervals (SDNN), the differences between the longest and shortest NN intervals, and the differences between nocturnal and diurnal heart rate ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European

Society of Cardiology and the North American Society of Pacing and Electrophysiology," 1996).

Frequency domain measures are used to calculate HRV in both short-term 2-5 minute and long term recordings, i.e. 24 hours (Akselrod, et al., 1981). However, traditionally, spectral analyses are done in laboratory studies for short term recordings of HRV (Stein, Bosner, Kleiger, & Conger, 1994; Stein, Domitrovich, Huikuri, & Kleiger, 2005). There are various spectral methods used to calculate the frequency domain of the HRV. Power spectral density (PSD) indicates how power distributes as function of frequency (Akselrod, Gordon et al. 1985; 1996). Three main spectral components of HRV are high frequency power (HF, 0.15-0.40 Hz), low frequency power (LF, 0.04-0.15 Hz), and very low frequency power (VLF ≈ 0) (Luczak & Laurig, 1973).

Research by numerous investigators has shown that HF reflects the vagal or parasympathetic control of the heart (Bloomfield, et al., 2001; Brown, Gee, Olah, Docker, & Taylor, 1992; Luczak & Laurig, 1973; Stein, et al., 1994; Stein, et al., 2005). LF power is modulated by baroreflexes and a combination of sympathetic and parasympathetic efferent impulses on the SA node (Billman, 1986; Bloomfield, et al., 1997; Kleiger, et al., 2005; Stein, et al., 1994). Therefore, LF represents both sympathetic and parasympathetic control of the heart. However, some researchers indicate that LF power and changes in LF predominantly represent the sympathetic regulation or sympathetic activation of the heart (Malliani & Pagani, 1991; Malliani, Pagani, Lombardi, & Cerutti, 1991; Montano, et al., 1996; Pagani, et al., 1991; Piccirillo, et al., 2007). Even though various mathematical manipulations of LF power have been used to better assess the sympathetic control of the heart (normalization of LF power, use of the

ratio LF/HF; Kleiger et al., 2005), they have produced mixed results. For example, the increase in LF/HF was misinterpreted as an increase of sympathetic activity when the changes were actually due to vagal withdrawal and the subsequent decrease in HF power (1996; Kleiger, Stein et al. 2005).

In summary, HRV represents a rhythmic fluctuation of the heart rhythm. These fluctuations are evident at rest, standing up, at awakening, and during mental and physical challenges, and they represent the autonomic balance of the cardiac control by both branches of the ANS. The frequency domain of HRV is usually used for short-term recordings. HRV variability and its components reflect the sympatho-vagal regulation of the heart by the ANS. Research indicates that HF represents the vagal activity on the heart muscle.

Predictive value of HRV for SCD. Reduced HRV is thought to reflect an imbalance of the autonomic regulation of the heart and can serve as a risk factor or predictor of future adverse cardiac events, including. SCD (Carney, et al., 2000; Lampert, et al., 2002; Lewis, 2005; Mainardi, Bianchi, & Cerutti, 2002). Such an imbalance is hypothesized to be caused by reduced parasympathetic tone on the SA node and/or an increase in sympathetic firing (Lewis, 2005; Lombardi, Malliani, Pagani, & Cerutti, 1996; Stein & Reddy, 2005). This imbalance is especially heightened in CHD patients. For example, the less variable the heart signals are, the greater the risk of post- MI mortality from re-infarction or fatal arrhythmias due to ventricular tachycardia (1996; McMillan 2002). Wolf et al. (1978) observed two groups of patients (n = 176) after acute

MI. Patients with reduced R-R intervals or reduced sinus arrhythmia (n = 73) had higher in-hospital mortality than patients with normal sinus arrhythmia (n = 103).

A series of studies have examined the prediction of overall cardiac mortality in post-MI patients based on reduced HRV, decreased ejection fraction (proportion of blood pumped out by the ventricle with each beat), and the frequency of ventricular ectopy (Farrell, et al., 1991; Odemuyiwa, et al., 1993). These studies found that the predictive value of reduced HRV was comparable to that of left ventricular ejection fraction. However, in some studies HRV was a better predictor than ejection fraction for SCD and ventricular tachycardia in post-MI patients (Odemuyiwa, et al., 1993). Pozzati et al. (1996) analyzed 24 hour ECG tapes of 8 patients with CHD who died suddenly and 24 hour tapes of the patients with CHD who did not have life-threatening arrhythmias. Patients who subsequently had SCD had a significant decrease in HRV 5 minutes before the lethal outcome, and the control group did not exhibit such a marked decrease in HRV (Pozzati, Pancaldi, Di Pasquale, Pinelli, & Bugiardini, 1996). The decrease in total HRV power was followed with ST segment depression, representing myocardial ischemia, and then sudden cardiac death (1996; McMillan 2002). These data suggest that sympatho-vagal imbalance may trigger fatal arrhythmias during acute ischemia and result in SCD in CHD patients (1996; McMillan 2002). Other studies found a reduction in HF power and an increase in LF and LF/HF at rest and especially during stress in post-MI patients (Kamath & Fallen, 1991; Lombardi & Malliani, 1992). These changes may indicate the shift of sympatho-vagal balance towards sympathetic influence ("Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and

Electrophysiology," 1996). In addition, the changes of the spectral profile of HRV in acute post-MI patients are similar to those observed in heart failure patients or heart transplant patients (Arora, et al., 2004). The decreases in HF and increases in LF and LF/HF ratio in acute post-MI patients very likely reflect the diminished responsiveness of the heart or SA node to parasympathetic stimulation (Malliani, Lombardi, & Pagani, 1994; Malliani, Lombardi, Pagani, & Cerutti, 1994) or persistently high sympathetic tone on the SA node (Malik & Camm, 1993; Malliani & Montano, 2004).

It is important to note that large studies such as the European Myocardial Infarction Amiodarone Trial (EMIAT, N =743) and Autonomic Tone and Reflexes after Myocardial Infarction (ATRAMI, N = 1139) found that HRV was predictive of future cardiac death in post-MI patients, however it was not predictive of future arrhythmic events. This discrepancy in findings may be attributed to the definition of SCD, which includes not only patients with arrhythmic events, but also patients with re-infarction and other CVD (Domanski, et al., 2003; Greene, et al., 1989). Because not every SCD is an arrhythmic event, the superiority of HRV in predicting future arrhythmic events is questionable.

Summary. The autonomic imbalance of heart regulation expressed as a decrease in parasympathetic and/or an increase in sympathetic tones has been linked to adverse cardiac events. Delayed heart rate recovery and decreased high frequency of heart rate variability have been proposed as markers that reflect vagal modulation on the heart. However, their ability in predicting future arrhythmic events in cardiac and asymptomatic patients is still questionable.

***Myocardial substrate.*** Myocardial substrate (areas of jeopardized myocardium) is another component of the lethal triad, which may favor the conditions necessary for sudden cardiac death. These changes in myocardium usually relate to pathological, non-transient changes in heart morphology which could be measured through functional and morphological assessments such as decreased ejection fraction, wall motion abnormalities, non-sinus rhythm (AF), interstitial fibrosis, and scar tissue (Zareba & Moss, 2003). In particular, the predictive value of the left ventricular ejection fraction (LVEF) as a marker of SCD will be reviewed because EF is one of the oldest and most widely used risk stratifiers and one of the strongest predictors of poor outcomes in cardiac patients (Rouleau, Shenasa et al. 1990; Becker, Pepine et al. 1996)

*Ejection fraction.* Ejection fraction (EF) is the fraction of diastolic blood volume pushed out of a ventricle with each heart contraction or with each heart beat. The expression “ejection fraction” can be applied to both the right (RVEF) and left ventricles (LVEF). The amount of blood in a ventricle prior to a contraction is called “end-diastolic volume”. Similarly, “end-systolic volume” is the amount of blood left in a ventricle at the end of contraction. Stroke volume is the difference between end-diastolic and end-systolic volumes, or the volume of blood ejected in each beat. The fraction of the end-diastolic volume ejected in each beat is called the ejection fraction (Guyton & Hall, 2000).

EF = SV/EDV = EDV - ESV/EDV

Healthy individuals have ejection fractions greater than about 55% (Bigger & Bloomfield, 2007). Damage to the muscle of the heart, such as MI or cardiomyopathy, diminishes the heart's capacity to eject blood and thus reduces ejection fraction. Reduced ejection fraction is usually associated with poor clinical prognosis, and mortality increases exponentially as the EF falls.

Measures of EF. Ejection fraction can be measured by several techniques, including echocardiography and radionuclide ventriculography (Rozanski et al, 1982; (Guyton & Hall, 2000). The volumes of the heart's chambers are measured during the cardiac cycle. Ejection fraction is then calculated by dividing the stroke volume by the end-diastolic volume. Other methods of measuring ejection fraction include fast scan cardiac computed axial tomography (CT) imaging, cardiac magnetic resonance imaging (CMR), gated single photon emission computed tomography **(**gated SPECT**),** and the Multiple Gated Acquisition Scan (MUGA). SPECT provides three dimensional representations of the heart chambers. This information is usually presented as cross sections of the patient’s heart. A MUGA scan involves the injection of a contrast agent into the blood and imaging its flow through the left ventricle. It provides a movie-like image of the beating heart and allows for determination of the health of the heart’s major pumping chambers (e.g. ventricles). There is a growing body of research comparing the accuracy and advantages of the methods in identifying the EF (Martin, Graham, Kao, & Bashein, 1989; Mohan, et al., 2004; Soriano, et al., 2008). In the present study, the SPECT technique was used in order to quantify EF. SPECT has the unique ability to provide assessment of both myocardial perfusion (ischemia) and the function of left

ventricle (e.g., ejection fraction) (Ramachandruni, et al., 2006). In addition, it has high reproducibility (Verberne, Dijkgraaf, Somsen, & van Eck-Smit, 2003; Yoshikai, et al., 1999), as well as high correlations with other measures of ischemia (e.g., ventriculography, echocardiography) (Choragudi, et al., 2001). See Ischemia or Methods sections for more details regarding the application of this measure.

Predictive value of EF for SCD. Research shows that there is a progressive increase in cardiac mortality as left ventricular ejection fraction (LVEF) falls below 41% (Bigger & Bloomfield, 2007). A left ventricular ejection fraction (EF) of 35% or less increases the absolute risk of sudden death, however values in a range of 35 and 40% raise concern in the accuracy of the identification of the patients at risk (Piccirillo, et al., 2007). Note, LVEF fraction does not identify the mechanism of SCD as arrhythmic or not, it simply predicts both arrhythmic and non-arrhythmic sudden cardiac deaths (Buxton, 2005b).

Reduced LVEF was a major eligibility criterion for enrolling patients into several randomized clinical trials of ICD prophylaxis. In these trials, high risk was defined by low ejection fraction, usually below 31%, and additional risk stratifiers, including non-sustained VT, and inducible VT and VF (Moss, Hall et al. 1996; Moss, Daubert et al. 2002; Buxton 2005; Buxton 2005). Overall, the results of these trials demonstrate the efficacy of ICD therapy as the best prevention for SCD (Moss, Hall et al. 1996; Moss, Daubert et al. 2002; Buxton 2005; Buxton 2005) in low EF patients.

However, results from these studies do not suggest that LVEF alone is an optimal criterion for implanting an ICD. For example, the MADIT investigators restricted entry

into that trial by requiring an EF of 35%. MADIT patients with an EF of 26% to 35% did not exhibit improved survival with an ICD when compared with those randomized to conventional therapy, but patients with an EF <26% did exhibit significantly improved survival with an ICD (Moss, Fadl et al. 2001). In the Maastricht Circulatory Arrest Registry study, cases of sudden circulatory death were studied in the age group of 20 to 75 years over 4 year period encompassing 1 January 1997 and 31 December 2000 (Gorgels, Gijsbers, de Vreede-Swagemakers, Lousberg, & Wellens, 2003). 268 of 492 cases (54%) did not have any cardiac history, including low LVEF (Gorgels, et al., 2003). In another study, individual patient data from the AVID study, the Cardiac Arrest Study Hamburg (CASH) and the Canadian Implantable Defibrillator Study (CIDS) were merged into a master database in order to evaluate the benefits of ICD therapy (Connolly, Hallstrom et al. 2000). This study showed that ICD therapy, in comparison to drug therapy, is only beneficial for patients whose EF is below or equal 35%. Only one third of the patients with SCD had EF lower than 31%. These findings provide evidence that EF is not a sensitive enough marker to solely identify patients at risk of SCD (Bigger & Bloomfield, 2007).

It is more difficult to discuss the specificity of EF, since the mechanism of SCD may be attributed to arrhythmic and non-arrhythmic causes. Not all sudden deaths result from primary arrhythmic events. However, most of sudden deaths in patients who are not at end-stage heart failure can be attributed to arrhythmias (Buxton, 2005a). If malignant arrhythmias are not a primary mechanism of death and the reduction of mortality cases due to ICD therapy should not differ from the reduction of total mortality (Buxton et al., 2005). If this is the case, the assumptions can be made that patients with lower EF will

have higher likelihoods of arrhythmic events; however, the previously discussed literature shows quite opposite results. This argument leads one to conclude that EF as an isolated predictor of SCD does not have very high specificity. The Maastricht Circulatory Arrest Registry study also demonstrated that the negative predictive accuracy of LVEF is modest (Gorgels, et al., 2003).

Summary. The research indicates that reduced EF is related to poorer cardiac prognosis and increased risk of SCD. However, it is not clear which cut-offs for EF should be used to stratify the risk of SCD. The specificity, sensitivity, and negative prognostic value of EF as a marker of SCD are not accurate.

**Mental Stress**

***The stress response.*** Stress can be defined as a process by which environmental events challenge or threaten the organism. Definitions can be based on the presence of external stressors (e.g., death of a spouse, noise), the individual's responses (physical, psychological, emotional, and behavioral), and the physiological or health consequences of stress (e.g., impaired immune system function, atherosclerosis, depression, and sudden cardiac death) (J. R. Kaplan, Pettersson, Manuck, & Olsson, 1991; M. S. Kaplan, Pratley, & Hawkins, 1991; Lucini, Mela, Malliani, & Pagani, 2002; Rozanski, Blumenthal, Davidson, Saab, & Kubzansky, 2005).

Stress has been defined as a state of threat to the organism's homeostasis (Cannon, 1957). Adaptation to stress confers a survival advantage (McEwen, 2002). Responses of the autonomic nervous system, the hypothalamic–pituitary–adrenocortical

(HPA) axis, and the cardiovascular, metabolic, and immune systems protect the body by responding to internal and external stressors engendering successful adaptation to the challenge (McEwen, 1998; Selye, 1975a). Thus, physiological stress responses involve the sympathetic nervous system (SNS) and the HPA axis (Cannon, 1957; McEwen, 1998; Selye, 1954a).

*Physiology of the stress response.* Stressful environmental stimuli trigger the release of corticotrophin releasing factor (CRF), which causes the release of adrenocorticotrophic hormone (ACTH) and cortisol into the blood stream (Frankenhaeuser & Rissler, 1970; Mason, et al., 1961). The increased secretion of cortisol helps mobilize stored energy in response to perturbation, therefore increasing the individual's chance for surviving or enduring the physical challenge (McEwen, 1998).

In addition, the adrenal medulla receives messages from the brain through the sympathetic nerves. Sympathetic activation constitutes the primitive “fight-or-flight” response that prepares the organism for the intense exertion necessary for survival in the face of a threat (Selye, 1954b). In response to the stimulation of the sympathetic nervous system (SNS), the adrenal medulla releases epinephrine and norepinephrine (Frankenhaeuser, Mellis, Rissler, Bjorkvall, & Patkai, 1968; Howley, 1976). The physiological responses to catecholamines include increases in blood pressure, peripheral resistance, HR, and cardiac output (Guyton & Hall, 2000). SNS activation is balanced by the activation of the parasympathetic branch (PNS) of the autonomic nervous system. However, chronic activation of the SNS may shift the autonomic balance towards the sympathetic system (McEwen, 2004).

Adaptation to stress depends on both the ability to respond to stress and the ability to cope with the stressor. For example, predictability and control, or their perception, can reduce SNS and HPA axis activation during a stress event (Frankenhaeuser, 1978). The evaluation of the stressful situation or event by the individual in terms of harm and resources to cope (appraisal) can also modify SNS and HPA axis responses to stress (Lazarus, 1984, 1992; Mason, 1968a, 1968b).

Activation of the SNS and HPA axis prepares the organism for the vigorous motor activity needed to flee a dangerous situation or fight a threat in one's environment. In today's modern society, however, stressors are more likely to be psychological, not physical (Glass & Singer, 1972). Response to a psychological challenge involves responses similar to a physical challenge (i.e., increases in ACTH and cortisol secretion, sympathetic activity, catecholamines, heart rate, blood pressure, and peripheral vascular resistance). However, there are differences in responses to psychological stress versus responses to physical stress. Psychological stress is usually not tied to an increased metabolic demand, as is the response to physical stress, and may not have a definable beginning or end (Krantz & McCeney, 2002; McEwen, 2004). In addition, there are differences in autonomic nervous system activation and cardiovascular system responses. These differences will be discussed in detail in upcoming sections. Frequent activation of the HPA axis with associated increases in cortisol and sympathetic activation in response to psychological stressors have been linked to visceral obesity, hypertension, hyperlipidemia, and insulin resistance (Hautanen & Adlercreutz, 1993; Rosmond & Bjorntorp, 1998; Rosmond, Dallman, & Bjorntorp, 1998) and to an increased risk for

chronic conditions such as cardiovascular disease, diabetes, and atherosclerosis (Bjorntorp, 1997; Karasek, et al., 1988; McEwen, 2004).

***Mental stress and CVD.*** Research has shown that acute and chronic stress may contribute to the development and progression of various forms of CVD (Krantz and McCeney 2002; Rozanski, Blumenthal et al. 2005; Holmes, Krantz et al. 2006). For example, evidence from epidemiological studies has shown that mental stress may be a risk factor for the development of atherosclerosis, independent of other risk factors, such as high blood pressure, cholesterol, gender, age, and smoking (De Backer, et al., 2004; J. R. Kaplan & Manuck, 1999). Iribarren et al. (2000) studied a general population, with approximately equal representation of males versus females and Caucasians versus African Americans. Individuals who reported chronic anger and hostility were at elevated risk for developing atherosclerosis. Wang et al. (2006) followed a cohort of 80 women for three years and evaluated levels of stress exposure, mainly job stress and job satisfaction, and the progression of atherosclerosis. Women with high levels of stress had significant disease progression compared to those with low levels of stress. In addition, coronary artery changes showed regression in women who were satisfied with their marriages and work (Wang, Mittleman, Leineweber, & Orth-Gomer, 2006). These associations were independent of the disease severity, age, smoking, hypertension, and high density lipoprotein (HDL).

*MI and mental stress.* Several studies have explored the relationship of stressful life events to the occurrence of acute cardiovascular events, such MI and SCD. For

example, after the Northridge earthquake, a mail survey of more than 100 hospitals in the region showed that the number of MIs the week after the disaster was 201 in comparison to 149 the week before the earthquake (Leor & Kloner, 1996). Analysis of earthquakes in Hanshin-Awaji (Japan), Greece, and Australia also showed increases in MI rates after the incidents (Trichopoulos, Katsouyanni et al. 1983; Dobson, Alexander et al. 1991; Suzuki, Sakamoto et al. 1995). In the early stage of the Gulf War, the incidents of acute MI and SCD increased in an area close to missile attacks (Meisel, et al., 1991). National surveys in Israel showed a 58% increase in total mortality on the first day of the attacks and the majority of death was due to MI or SCD (Kark, Goldman, & Epstein, 1995). There was an increase in MI hospital admission in England during the 1998 World Cup against Argentina (Carroll, Ebrahim, Tilling, Macleod, & Smith, 2002). Moller et al. (1999) conducted a case-crossover study of MI patients. The results showed that patients who were exposed to an anger provoking event one hour prior to MI were 9 times more likely to have a heart attack in comparison to patients who were not exposed to anger provoking episodes.

*SCD and mental stress.* Acute psychological disturbances have been linked to life-threatening arrhythmias and SCD. For example, twenty-five of 117 cardiac patients experienced acute emotional disturbances within 24 hours prior to having ventricular arrhythmias (Reich, DeSilva, Lown, & Murawski, 1981). Smith et al. (2007) measured diary-reported stress in 80 post-MI patients undergoing 24-hour Holter monitoring. Mean diary-reported stress was positively associated with arrhythmic activity (ventricular ectopy) in the study. Shedd et al (2004) studied 132 ICD patients' records. In the 30 days

after the attack on the World Trade Center Twin Towers, 11 of the patients studied experienced ventricular tachycardia, compared to only 5 patients in the 30 days prior to the attack. The difference represents a 2.8 fold risk increase. In another study investigators interviewed relatives of 100 men who died suddenly (Myers & Dewar, 1975). They compared the interview results from relatives of patients with SCD with interview results collected from 100 men admitted to the hospital after MI. Patients did not differ in their coronary risk profiles. However, those who had sudden death were significantly more likely to have recieved moderate or severe stress in the 30 minutes prior to onset of death in comparison to MI patients (23% versus 8%).

***Mental stress in laboratory studies.***

*Pathophysiological response to mental stress.* As described above, research shows a link between the development of CVD and mental stress. In past decades, mental stress has been used in laboratory studies to investigate the mechanisms linking emotional triggers to the development or worsening of CVD. Primary research focused on the exaggerated responses of the cardiovascular system to mental stress, mental stress induced ischemia, and mental stress induced arrhythmias.

When the brain perceives acute mental stress as a challenge, it activates the sympathetic nervous system and inactivates the parasympathetic influences in order to prepare the organism for exertion (Mason, 1968a; McEwen, 2004; Selye, 1975c). This sympathetic nervous system activation includes, through impact on beta-adrenergic receptors, an increased contractility of the myocardium and an increase in heart rate, followed by an increase in cardiac output, and finally an increase in vascular resistance

through small artery constriction (Strike, et al., 2004). This results in increased systolic and diastolic blood pressures. Myocardial oxygen consumption is determined by heart rate, contractility, and wall stress as determined by systolic pressure and chamber radius. Therefore, sympathetic stimulation also increases myocardial oxygen consumption significantly (Guyton & Hall, 2000). Thus, in the face of an acute stressor, cardiac demand on the oxygen supply increases (Sherwood & Turner, 1995; Soufer, 2004).

Furthermore, the sympathetic responses of the heart to stress are accompanied by a decrease in vagal tone (Soufer, 2004). Vagal withdrawal during stress and a shift of the autonomic balance towards sympathetic influence leads to an increase in myocardial oxygen consumption, hemodynamic resistance, myocardial contractility, and a decrease in the arrhythmic threshold of the heart. These changes in cardiovascular functioning may lead to myocardial ischemia, fatal arrhythmias and MIs (Krantz, Kop, Santiago, & Gottdiener, 1996; J. E. Muller, Tofler, & Edelman, 1989).

*Stress reactivity.* Mental stress is a multi-faceted process involving the environment, individual characteristics (e.g. personality and emotions), individual experiences and coping, and physiological responses to stress (cardiovascular, neuroendocrine, and autonomic) (Soufer 2004; Holmes, Krantz et al. 2006). The complexity of stress and stress responses and their association with individual personality factors resulted in an array of research focusing on the relationships of acute cardiovascular responses to CVD and personality factors to CVD. For example, it has been hypothesized that individuals with exaggerated cardiovascular responses (e.g., blood pressure, heart rate changes) during mental stress may be at greater risk for the

development of cardiovascular syndromes such as hypertension or coronary heart disease than those exhibiting relatively small responses (Krantz & Manuck, 1984; Krantz & McCeney, 2002).

Research shows that humans and animals with exaggerated cardiovascular responses to mental challenges are at a higher risk of subsequent coronary events and CHD compared to those with normal cardiovascular responses to mental challenge. Manuck and colleagues (1997) found that cynomolgus monkeys who exhibited heightened cardiovascular reactions to psychological stress had higher levels of atherosclerosis compared to monkeys with normal stress reactivity. Matthews et al. (2006) found that exaggerated SBP response of young human adults while playing a video game predicted increased coronary calcification 13 years later. In another study, individuals at elevated risk for essential hypertension exhibited higher cardiovascular reactivity, HR, and BP responses during a public speaking task than did individuals with a lower risk for essential hypertension (al'Absi & Wittmers, 2003).

Stress reactivity in CHD patients**.** The relationship between exaggerated cardiovascular responses to acute stress and subsequent adverse cardiac events may be more evident in patients with clinical or sub-clinical CHD. For instance, individuals with pre-clinical CHD who had exaggerated cardiovascular responses to mental stress, but did not have known CHD, exhibited higher levels of cardiac ischemia during exercise in comparison to individuals with normal levels of stress reactivity (Kral, et al., 1997). Post-MI patients with exaggerated cardiovascular reactivity to mental stress were at increased risk for the development of re-infarction or stroke, during the 39 to 64 months after acute

MI in comparison to post-MI patients with normal cardiovascular reactivity (Manuck, Olsson, Hjemdahl, & Rehnqvist, 1992). In another study, the relationships between hemodynamic reactivity and myocardial ischemia during laboratory mental stress were evaluated in 39 CAD patients and 12 controls (Krantz, et al., 1991). Results revealed that SBP levels during a mental stress task and SBP reactivity were highest for the severely ischemic group and lowest for controls (Myers & Dewar, 1975). *Characteristics of mental stress induced ischemia.* Even though atherosclerosis, MIs, and SCD due to lethal arrhythmias are of primary importance in prevention of CVD and mortality, the laboratory and field studies of mental stress induced ischemia may help in understanding the pathophysiological mechanisms by which mental stress leads to arrhythmic events.

Furthermore, exercise testing occurs in controlled environment with gradual increases in demand. In daily life, individuals are faced with a vast variety of mentally stressful challenges, and most of these triggers occur unexpectedly and in an uncontrollable manner (Krantz, et al., 1996). Ischemia during mental stress and daily life is different from the ischemia elicited during exercise testing in the laboratory. For example, Holter monitoring shows that out of hospital ischemia occurs during a wide variety of physical and mental activities, not just during strenuous exercise (Krantz, et al., 1996). Out of hospital ischemia is predominately silent and occurs at a lower HR than exercise induced ischemia (Krantz, et al., 1996). This may be because systemic vascular resistance decreases during dynamic exercise but increases during mental stress, leading to a higher afterload and which in return increases the likelihood of ischemia (Goldberg, Becker et al. 1996; Soufer 2004).

The sympathetic hyperactivation produced by mental stress may lead to wall motion abnormalities even in healthy men and women (Rozanski, Berman et al. 1982; Krantz, Santiago et al. 1999; Ziegelstein 2007). Becker et al. (1996) studied 29 healthy individuals with no CVD, no coronary risk factors, and a negative exercise test. Healthy volunteers underwent the Stroop Color-Word test and simulated public speaking. There were increases in BP, HR, stroke volume, EPI and NE. However, despite the increase in sympathetic activation and left ventricular (LV) volume, the EF decreased in 12 individuals (Becker, Pepine et al. 1996). Three of the five individuals with EF decreases over 8% developed regional wall motion abnormalities. The decrease in EF could be attributed to an afterload increase during mental stress.

In general, mental stress ischemia has been reported in 20-70% of patients with exercise-induced ischemia (Krantz, et al., 1999). However some research shows that mental stress testing may induce ischemia in CVD patients with negative (no ischemia) exercise testing (Ziegelstein, 2007). Ramachandruni et al. (2006) investigated 27 CVD patients with negative ischemia testing to exercise or pharmacological agents. All patients performed a speaking task involving role-playing a difficult interpersonal situation. Six patients showed evidence of an ischemia, measured by using SPECT, during the mental stress task. None of the subjects had chest pains or ECG detected (ST segment depression) ischemia (Ramachandruni, et al., 2006).

In addition, there is also a circadian rhythm to myocardial ischemia (Krantz, et al., 1996), such that it is higher during the early morning in comparison to the evening (Krantz, Kop et al. 1996; Krantz, Santiago et al. 1999).

*Mental stress induced arrhythmias.* As described, the primary cause of SCD is malignant arrhythmias (VT and VF). The mechanism leading to malignant arrhythmias during mental stress may differ from the pathways employed during a physical challenge. For example, the presence of ischemia may lead to the dispersion of repolarization processes, leading to a higher likelihood of re-entry, thereby lowering arrhythmic threshold. However the nature of ischemia during mental stress is different in comparison to exercise. It occurs at a lower HR, with higher afterload, decreased EF and it is usually silent. Autonomic imbalances of heart regulation may also lower the arrhythmic threshold. Mental stress, in comparison to exercise stress, does not have a clear beginning or end. The research also shows that mental stress induces asymmetric membrane activity in the sympathetic branch of the nervous system, leading to a dispersion of repolarization in the heart muscle (Critchley, et al., 2005). Furthermore, during mental stress there is a greater increase in Epi, in comparison to NE, and exercise stress leads to greater increase in NE, in comparison to Epi (Dimsdale and Moss 1980; Paavonen, Swan et al. 2001). Abnormalities in the myocardial substrate are also contributors to the likelihood of arrhythmias. Research shows that during mental stress there is decreased EF due to increases of afterload (Becker, Pepine et al. 1996; Goldberg, Becker et al. 1996; Jain, Shaker et al. 1998). This may exacerbate the ischemia of the myocardium and further increase the likelihood of arrhythmia.

Several studies have investigated the link between mental stress and malignant arrhythmias. A case-crossover study of 43 ICD patients with documented recurrence of spontaneous ventricular arrhythmias showed that 24% of the patients had some type of mental stress within an hour of or during the events (Fries, Konig, Schafers, & Bohm,

2002). Stopper et al (2007) investigated ICD recordings and the structured diary entries of 24 patients. Anger triggering events were more likely to have premature ventricular contractions (PVC) and were more likely lead to ventricular arrhythmias in comparison to non-anger triggering events, including physical activity (Stopper, et al., 2007).

In another study, patients with ICDs were given diaries to record levels of anger and physical activity (Lampert, et al., 2002). A total of 107 confirmed arrhythmic reports from 43 patients required recurring ICD therapies or shocks. Anger during a period of 0-15 minutes prior to administration of the shock occurred 15% of the time, while anger was reported only 3% of the time during the control period one week later (OR = 1.83, CI = 1.04-3.16).

Eighteen ICD patients were subjected to noninvasive programmed stimulation and the mental stress tasks of mental arithmetic and anger recall. VT was induced in 10 patients during mental stress. VT, induced during mental stress in 5 patients, was difficult to terminate and required repeated shocks in comparison to VT induced during rest-awake time (Lampert, Jain, Burg, Batsford, & McPherson, 2000). Furthermore, the VT during mental stress occurred in the absence of ischemia. Taggart et al (2005) further investigated mental stress induced arrhythmic vulnerability. Twelve healthy volunteers underwent continuous ECG recording during various mental stress tasks. In the absence of ischemia, the results showed subsequent increases in T-wave, LF and LF/HF ratio, indicating possible increases in the amount of sympathetic modulation of the heart. Researchers concluded that arrhythmic vulnerability during mental stress may increase not only due to ischemia, but also due to the autonomic imbalance leading to an increase in the dispersion of repolarization (Taggart, et al., 2005).

***Summary.***Research has shown a link between acute mental stress and subsequent coronary events (e.g., MI or SCD). This relationship may be attributed to the activation of the sympathetic nervous system and the deactivation of the parasympathetic nervous system leading to increased myocardial demand. This increased demand on the myocardium in patients with known or sub-clinical CHD can be detrimental and may lead to an increase in myocardial ischemia and lethal arrhythmias. In addition mental stress may influence cardiovascular and autonomic nervous systems differently in comparison to exercise. In modern times, people are more likely to experience mental stress; therefore, it is important to investigate the differences between exercise and mental stress. Accordingly, it will be important to evaluate the use of mental stress as a tool for identifying populations at risk for malignant arrhythmias

***Stress Response during Physical and Mental Challenges.*** As was reviewed above physical and mental challenges produce an overall increase in the activity of the sympathetic nervous system and a decrease in parasympathetic activity. This section will discuss the differences in the activation of the sympathetic nervous system and CV responses between physical and mental challenges

During dynamic exercise, the increased demand for oxygen by working muscles leads to the activation of the sympathetic nervous system. This results in an increase in the heart rate and a small or moderate increase in systolic blood pressure, cardiac output. Preload, the degree to which the myocardium is stretched before it contracts also increases with exercise. Increase in preload leads to stronger contraction of the

myocardium and subsequent increase in cardiac output. SNS activation also results in a decrease of peripheral vascular resistance and afterload, the resistance against which blood is expelled. During exercise there is a redistribution of blood flow towards actively working muscles. In the diseased heart the increased demand on the myocardium during exercise may lead to or exacerbate myocardial ischemia and malignant arrhythmias. Therefore, exercise testing has been traditionally used in order to identify patients at higher risk of adverse cardiac events.

During mental stress, activation of the SNS, as was described before, also results in increased systolic and diastolic blood pressures, increases in HR, and increased cardiac output (Strike & Steptoe, 2002). However, the increases in these variables are lower in comparison to exercise and there are increases or no change in systemic vascular resistance (SVR). The increase in SVR during mental stress is especially true in case of patients with CVD (Jain, et al., 1998). The increase in SVR results in increased afterload, causing increase in work expenditure by the myocardium (Goldberg, Becker et al. 1996; Jain, Shaker et al. 1998). Therefore the increase in afterload may be followed by decrease in EF (Goldberg, Becker et al. 1996; Strike and Steptoe 2002).

In addition, differences may exist between plasma epinephrine (Epi) and norepinephrine levels (NE) depending on the type of the challenge. During exercise and mental stress there is an increase in Epi and NE. However exercise shows more pronounced increases in NE levels in comparison to Epi levels (Dimsdale and Moss 1980; Paavonen, Swan et al. 2001). In contrast, mental stress leads to greater increases in Epi levels (Goldberg, Becker et al. 1996), than NE levels in plasma. These findings

suggest that mental stress may have greater impact on the adrenal system, and exercise may lead to increased activation of the central sympathetic nervous system.

There are various studies comparing cardiovascular responses to mental and physical challenges in patients with CVD. Mazzuerro et al (1991) found an increase in heart rate, cardiac output and blood pressure in 81 post-MI patients during exercise and during mental arithmetic. These variables increased to a greater extent during exercise than during mental stress. During mental stress, there was a slight increase in systemic vascular resistance and a decrease in mean stroke volume. In comparison, during the exercise challenge, there was a noted decrease in systemic vascular resistance and an increase in mean stroke volume. Furthermore, during exercise testing, 33% of the patients had ST segment depression. No patient showed ST segment depression during the mental arithmetic tasks.

Rozanski and Krantz (1991) studied ventricular responses to mental stress testing in CAD patients. Twenty-three of the 39 CAD patients demonstrated wall motion abnormalities during mental stress. The majority of these patients also demonstrated a 6% or greater reduction in the ejection fraction during mental stress. Wall motion abnormalities were mainly detected in patients with exercise-induced wall motion abnormalities. During mental stress, these abnormalities would occur in the absence of the chest pain and at lower heart rates than during exercise.

In another study, the hemodynamic (BP and HR) and ischemic responses (measured via SPECT) were compared during mental stress (anger recall) and bicycle ergometry in 58 patients with CAD. Of the 58 CAD patients, 22 had normal LV function ($EF \geq 50\%$), 16 had mild to moderate LV dysfunction ($30\% < EF < 50\%$), and 20 had

severe LV dysfunction (Holmes, et al., 2007). For the entire sample, heart rate ($p<0.001$) increased more with exercise than with mental stress, whereas diastolic blood pressure ($p<0.001$) increased more in mental stress than exercise and systolic blood pressure increased similarly for exercise and mental stress. There were no differences in hemodynamic responses and ischemic responses during mental stress across different EF groups (Holmes, et al., 2007).

Kop et al (2004) evaluated T wave alternans (TWA) responses during laboratory mental stress (anger recall and mental arithmetic) and bicycle ergometry in ICD patients ($n = 23$) and controls ($n=17$). TWA was assessed from ECG by modified moving average analyses. TWA increased during mental stress and exercise. TWA responses were higher in ICD patients in comparison to controls. TWA during mental stress occurred at lower heart rates and was not associated with ischemia and EF, while no such relationship was seen during exercise. The authors hypothesized that the pathophysiological mechanism of mental stress-induced arrhythmia may involve central and autonomic pathways of CNS that differ from exercise-induced arrhythmias (Kop, Krantz et al. 2004).

Dimsdale and Moss (1980) monitored plasma catecholamine levels in a group of subjects during exercise and public speaking. During exercise, NE levels increased 3 fold, whereas during public speaking Epi levels increased 2-fold (Dimsdale & Moss, 1980).

It is possible that use of both types of stressors (exercise and mental stress) may enhance risk-stratification for adverse cardiac events. For example, Krantz et al (1999) studied 79 CAD patients with exercise induced ischemia, during which patients were subjected to a mental stress task. During 3.5 years of follow-up, 28 of the patients had

adverse cardiac events including SCD and MI. Survival analyses indicated that patients with mental stress induced ischemia were more likely to have adverse cardiac events in comparison to patients with only exercise induced ischemia (Krantz, et al., 1999).

In sum, Research suggests that mental stress and exercise stress may affect the CV and autonomic nervous systems via different pathophysiological pathways. During exercise there is an increase in systolic blood pressure, heart rate, and preload, and a decrease in afterload and systemic vascular resistance. During mental stress, there is an increase in systolic and diastolic blood pressures, heart rate, and afterload. Systemic vascular resistance tends to stay the same or increase slightly. In addition, during mental stress, ischemia and T-wave alternans and arrhythmias occur at a lower heart rate.

**Relationships among Non-invasive Markers of Sudden Cardiac Death**

Several non-invasive factors have been proposed as markers in identifying patients at higher risk for SCD (e.g., TWA, QTVI, inducible ischemia, HRV, and HRR). However none of these markers is sufficient as independent predictors of future malignant arrhythmias. Currently TWA may be the leading risk stratifier for future VT; however, this marker does not assess the repolarization variability at lower levels of exercise (associated with lower heart rates), or variability at lower frequencies. Because TWA may exclude the identification of the patients who are at risk of arrhythmias due to repolarization dispersion at low frequencies, theoretically, the use of other markers (e.g. autonomic imbalance) with TWA may help to improve risk stratification by assessing pathways other than myocardial instability leading to SCD. The incremental prognostic value of MTWA and MMA, when combined with other methods of risk stratification, is

not clear. In addition, the relationships among various markers of SCD may differ depending on the type of the challenge (physical or mental) encountered by organism. For example, ischemia during mental stress is not associated with TWA. In contrary, ischemia during exercise is associated with TWA. This may be due to the pathophysiological response during mental stress having different pathways in comparison to exercise stress. Critchley et al (2005) have found that T-wave abnormalities (dispersion of repolarization) during mental stress were associated with asymmetry (reflected by regional cerebral blood flow) in midbrain activity. This asymmetry may lead to an imbalance in sympathetic cardiac left and right nerves activity. This imbalance in sympathetic nerve activity may lead to increased dispersion of the repolarization process reflected by changes in T-wave (Critchley, et al., 2005).

Several studies looked into relationships among non-invasive markers of SCD. Atiga et al (1998) investigated the ability of the QTVI to identify patients at risk of SCD or sustained VT. Ninety five cardiac patients presented for electrophysiological study (EPS). Measures of TWA alternans, total HRV, QTVI, VT infusibility at EPS, and EF were collected during atrial pacing. Patients were followed for an average of 24 months. The QTVI was the only clinical variable that identified patients with SCD (Atiga, et al., 1998). This study has two important limitations: 1) the researchers did not investigate the relationships among non-invasive markers of SCD, and 2) they did not study the relationships under exercise and mental stress.

Hohnloser et al (1998) compared the predictive value of left ventricular ejection fraction, heart rate variability, microvolt T-wave alternans (MTWA), baroreflex sensitivity, electrophysiology study (EPS) and QT dispersion in identifying ICD patients

at risk of arrhythmia. The results of multivariate Cox regression analysis demonstrated that TWA was the only significant independent risk factor for the appropriateness of the ICD therapy (Hohnloser, Klingenheben et al. 1998). However, this study did not investigate the relationships during mental stress. Furthermore, the study did not examine ischemia and heart rate recovery as possible predictors of arrhythmic vulnerability.

Lampert et al. (2005) studied thirty eight ICD patients. Patients receiving continuous ECG monitoring were subjected to two mental stress tasks (arithmetic and anger recall) (Lampert, et al., 2005). During mental stress, there was an increase in catecholamine levels, TWA, blood pressure, and heart rate and a decrease in HF power of HRV. There were no ischemic changes (indicated by ECG ST segment depression) during mental stress. Changes in TWA correlated with changes in HR, systolic BP, and catecholamine levels. There was no relationship between TWA and HF. In this study, the investigators did not examine the relationships between TWA and other non-invasive markers such as QTVI, heart rate recovery (HRR), and EF.

Kop et al (2004) evaluated T wave alternans (TWA), hemodynamic, ischemic and EF responses during laboratory mental stress (anger recall and mental arithmetic) and bicycle ergometry in ICD patients. TWA responses were higher in ICD patients in comparison to controls. TWA during mental stress occurred at lower heart rates and was not associated with ischemia and EF. While no such relationship was seen during exercise. However, the relationships among markers of autonomic imbalance (e.g, HF of HRV and HRR) and TWA were not examined.

**Rationale for the Proposed Study**

The majority of SCD occurs due to ventricular fibrillation and ventricular tachycardia (Zipes, et al., 1983). Zareba (2003) categorized triggering factors necessary for the occurrence of malignant arrhythmias and subsequent cardiac arrest into 3 domains: increased vulnerability of the myocardium to arrhythmias, autonomic nervous system imbalance, and changes in myocardial substrate. There is a need to determine a non-invasive marker or combinations of markers which may help identify patients at risk for SCD. According to recent literature, TWA seems to be one of the leading non-invasive parameters which may help to stratify the risk of SCD (Bigger and Bloomfield 2007; Narayan 2008). However, studies of its relation to other proposed markers (e. g. QTVI, HRV, HRR, ischemia, and EF) have produced conflicting results regarding which of these markers are best at predicting malignant arrhythmias and the extent to which these markers are correlated. Given that mental stress occurs frequently during daily life, there is a need to (1) understand how these markers interact during mental stress, (2) further elucidate the differences in risk markers levels during exercise and mental stress, and (3) examine how the relationships between these non-invasive risk markers might change under different types of stressors.

The purpose of the present study is to examine the relationships between TWA and (1) QTVI, another marker of arrhythmic vulnerability; (2) ischemia, a marker of arrhythmic instability; (3) ejection fraction, a marker of myocardial substrate changes; and (4) HF and HRR, markers reflecting the parasympathetic tone of the heart. The study also seeks to determine the amount of TWA variance explained by all markers of SCD (QTVI, HF, ischemia HRR and EF) together. Given that TWA occurs at a lower HR and does not usually relate to ischemia or EF during mental stress, the study also aims to

examine the relationships among these non-invasive markers under mental and exercise challenges.

**Specific Aims**

Main study objective: To assess the relationship between the non-invasive markers of myocardial vulnerability (QT variability and inducible ischemia), autonomic activity (HF and heart rate recovery), and myocardial substrate (EF) to TWA in ICD patients under two acute laboratory challenges (exercise and mental stress) known to elicit different cardiac and peripheral hemodynamic patterns in healthy individuals and patients with CVD vulnerable to arrhythmias. In addition, use of information on non-invasive markers collected during both challenges (exercise and mental stress) may be a better approach for risk stratification of patients at risk of future adverse events in comparison to use of information collected during only one challenge (Krantz, et al., 1999).

In addition to achieving these objectives, we examined the agreement between two different methods of TWA assessment. The data on MTWA (Cambridge Heart) due to methodological issues with MTWA assessment (see Methods section) was available only during exercise; therefore, the agreement between MTWA and MMA-TWA was examined during exercise.

Aim I: <u>To compare levels of T wave alternans, EF, ischemia, HF and HRR during mental stress versus during exercise in CVD patients with implantable defibrillators.</u>

Hypothesis I: Compared to mental stress, during exercise patients would have (1) higher levels of TWA, (2) faster HRR, (3) higher prevalence of ischemia, and (4) lower HF level.

Aim II (a): To assess the relationships between TWA and EF, HF, HRR, resting QTVI and ischemia during exercise and mental stress.

Hypothesis II (a): For the exercise challenge, ischemia and pre exercise resting QTVI would be positively related to MMA-TWA,

while EF, HRR and HF would be inversely related to TWA. As to the mental stress challenge, pre stress resting QTVI would be positively related to MMA-TWA, while EF, HRR and HF would be inversely related to MMA-TWA; and ischemia would not be associated with MMA-TWA.

Aim II (b): To compare individual associations of EF, HF, HRR, resting QTVI and ischemia to TWA between exercise and mental stress challenges.

Hypothesis II (b): It was hypothesized that individual associations between TWA and other markers of arrhythmic vulnerability would be stronger during exercise in comparison to mental stress challenge.

Aim II (c): To assess the collective effect of EF, HF, HRR, resting QTVI and ischemia on TWA during exercise and mental stress challenges.

Hypothesis II (c): It was hypothesized that the collective effect of the non-invasive markers to TWA would be stronger in comparison to relationships of each marker alone to TWA during both exercise and mental stress.

Aim II (d): To compare the collective effect of EF, HF, HRR, resting QTVI and ischemia on TWA during exercise to the collective effect of these markers on TWA during mental stress.

Hypothesis II (c): It was hypothesized that the collective effects of EF, HF HRR, resting QTVI and ischemia on TWA would be stronger during exercise than during mental stress.

## Methods

To test the proposed hypotheses, patient data from the Triggers of Arrhythmia in Defibrillator (TRIAD) study will be analyzed. The study began in 1997 and ended in 2004.

### Participants

In the TRIAD study, 102 patients were recruited from the Veterans Affairs Medical Center (Washington, DC), Arrhythmia Associates (Fairfax, VA), and Saint Francis Hospital (Roslyn, NY). The study included 18 patients with stable coronary artery disease (CAD), 57 patients with known coronary artery disease and arrhythmic vulnerability, and with implantable cardioverter defibrillators (ICDs), and 27 age and gender matched controls with less than a 5% likelihood of CAD. The primary goal of the TRIAD study was to investigate predictors of malignant cardiac arrhythmias in vulnerable patients in order to assess whether and in what manner stress-induced ischemia interacts with cardiac electrical instability in triggering SCD. For the purposes of the present study, data collected from the 50 CAD patients with ICDs will be analyzed.

ICD patients were identified from the Cardiology Services Department of the three participating recruitment centers. To be eligible for the CAD with ICD group in the TRIAD study, patients had to have had known CAD established by prior angiography or documented MI and have been implemented with ICDs capable of storing information about arrhythmias for which shocks were delivered, including time of day and R-R intervals, or electrocardiograms (ECG). Patients were excluded from the study if: 1) they were receiving amiodarone; 2) had primary diagnosis of cardiomyopathy; 3) had class IV

congestive heart failure; 4) had MI within the month prior to enrollment in the study; 5) had unstable angina; 6) had critical valvular pathology; 7) had chronic atrial fibrillation; and 8) had baseline abnormalities of the ST- segment, for example due to left ventricle hypertrophy or bundle branch block, as these abnormalities make the ECG ST-segment depression uninterpretable for ischemia. Patients were also excluded from the study if they had neurological or psychiatric disability which interfered with the ability to understand, respond, or consent to the mental stress task.

**Study Procedure**

The TRIAD study consisted of 3 phases: a laboratory session, a Holter monitoring phase, and longitudinal follow-ups. For the present study, only laboratory data will be analyzed. The procedure for laboratory testing is described below. The laboratory session included a mental stress session (MS) and an exercise stress session (EX) on consecutive days. To control for the effects of sequence, the order of the sessions was randomly determined for each patient at the first visit.

Phase 1: Mental Stress and Exercise Laboratory Challenges

Both challenges included 4 stages: preparation, rest, testing, and recovery. A blood pressure cuff was placed around the participant's joint effect arm to measure blood pressure (BP). Using techniques published by Nearing and Verrier (2003), a 15 lead ECG system was attached to the patient and a small intravenous catheter was placed in order to inject the radioisotopes used to measure heart perfusion. This period lasted approximately 20 minutes.

During the rest stage participants were asked to sit and relax for 15 minutes in a quiet room with dimmed lights. ECG was continuously recorded, and systolic and diastolic blood pressures (SBP and DBP respectively) and HR were taken every 90-second. During rest period first radioisotope (thallium) was injected.

***Mental stress.*** The mental stress day included two 3-4-minute mental stress tasks: anger recall and mental arithmetic. Both mental stress tasks were shown to elicit ischemia in 30% to 60% of CAD patients (Ironson, Taylor et al. 1992; Kop, Verdino et al. 2001). Patients were administered the anger recall task first. The anger recall task, based on a method used Ironson et al (1992), consisted of asking patients to recall a previously-experienced anger incident and to discuss the circumstances of this incident in front of members of the research team. The mental arithmetic task immediately followed. During the mental arithmetic task, via tape recorded voice, patients were asked to serially subtract 7 from a 4-digit number while being interrupted and urged to improve performance (Yeung, Vekshtein et al. 1991; Kop, Verdino et al. 2001). During both mental stress challenges the research team used standardized scripted protocols. The ECG was continuously recorded and SBP, DBP and HR were taken at 90-second intervals during the mental stress challenge. At the 2-minute point of the mental stress task, the second radioisotope (sestamibi) was injected.

Bicycle exercise. The exercise challenge consisted of bicycle ergometry. Bicycle exercise was performed by increasing the workload by 25 Watts in 3 minute stages from 25 Watts at onset of exercise, according to the standard CH2000 (Cambridge Heart) protocol (Kop, Krantz et al. 2004). Patients were asked to exercise until fatigue or until

85% of maximum HR, predicted based on the participant's age, was reached and maintained. After reaching 85% of max HR, exercise was continued for 1 minute with the resistance of the pedals held constant. Exercise was stopped if the patient experienced chest pain, serious arrhythmia, or ST segment depression more than 1 millimeter on the ECG.

During exercise the ECG was continuously recorded and SBP, DBP and HR were taken at 2 minute intervals. At peak exercise, participant reaching 85% of max HR, a radioisotope (sestamibi) was injected.

The recovery stage lasted 8-10 minutes after both exercise and mental stress challenges. During this stage, the ECG was continuously recorded and SBP, DBP and HR were taken at 2 minute intervals.

Patients were studied in the fasting stage during the laboratory visits. To optimize assessment of TWA, HRV, QTVI and ischemia, calcium antagonists and ACE inhibitors were withheld for 24 hours in 3 and 2 patients respectively. Six patients were tested while on therapy with calcium antagonists and 30 patients were tested while on therapy with ACE. Long-acting nitrates were withheld for 6 hours, and 11 patients were tested without discontinuing nitrates. Beta-adrenergic blocking agents were withheld for over 48 hours in 4 patients and 30 patients were tested without discontinuing beta-blockers.

The study was approved by the Institutional Review Boards at Uniformed Services University of the Health Sciences (where the study was based), University of Maryland Baltimore (where the laboratory sessions were conducted), and the participating recruitment centers. Written informed consent was obtained from all participants.

**Measures.** The table below displays summary of measures and their times of administration during lab testing.

| Laboratory stages: | Rest | Stress (EX&MS) | Recovery (EX&MS) |
|---|---|---|---|
| MMA-TWA | | X | |
| MTWA | | X | |
| QT variability Index (QTVI) | X | | |
| Ejection Fraction | X | | |
| High Frequency | | X | |
| Heart rate recovery | | | X |
| Ischemia | | X | |

***ECG.*** Continuous 15 lead (6 precordial, 6 limb leads and 3 orthogonal) ECGs were recorded using high-resolution silver-silver chloride electrodes (High-Res, Cambridge Heart, Inc., Bedford, Massachusetts). The ECGs were digitized at 1000 Hz with 16-bit resolution with CH2000. The digitized ECGs will be exported for offline analyses by a trained reader using a GE Medical Systems Workstation. ECG recordings will be used in order to derive data on TWA, QT variability index and HF of heart rate variability.

***T-wave alternans.***

*WA-MMA.* The complex demodulation of T-waves from digitized ECG recordings was evaluated using Modified Moving Averages analyses (MMA) software developed by Verrier and Nearing (2003). In a laboratory study, it was shown that MMA is capable of identifying post-MI patients who are at 4-fold or greater risk of malignant arrhythmias (Nearing & Verrier, 2003). The MMA method is less affected by changes in HR in

comparison to MTWA method and allows the collection of continuous data in moving subjects.

The following summarizes the procedures of MMA methods. Detailed description of the MMA method can be found in Nearing and Verrier, 2003. The first step in the MMA method removing the wander, premature and noisy beats from ECG recordings. The next step involves separating normal heart beats into odd (A) and even (B) groups and averaging the waveforms for each group (see figure 5D). The averaging algorithm in the MMA method is a function incorporating weights to limit the influence from single aberrant beats. The last step is the computation of TWA: the maximum differences in amplitude between the average odd-beat and the average even-beat complexes from the J point (i.e., the junction between the QRS complex and the ST segment) to the end of the T wave. In the present study, TWA was taken as the average of every 32 heart beats over 2 to 3 minute ECG segments during anger recall and stage 1 exercise. Anger recall part of the mental stress challenge was chosen for the MMA-TWA analyses, because based on previous research anger-induced TWA predicts future ventricular arrhythmias (Lampert, Shusterman et al. 2009).

TWA-MMA analyses of ECG were conducted use the GE Medical Systems Workstation. The software generates a report which summarizes results for each lead (see Figure 6 for an example). In the evaluation of the results several factors should be taken into consideration: level of noise during TWA, HR, and alignment of QRS templates. First, the level of noise has to be below the TWA amplitude in order to avoid artifacts in TWA assessment. The general rule of thumb in evaluating noise is that the signal (TWA amplitude) to noise (noise amplitude) ratio should be $\geq 1.2$, and that the noise should not

exceed 20 microvolt (μV). Secondly, at high heart rate, healthy individuals may also display high TWA; therefore, in order to increase the specificity of the test (i.e., rates of true negatives), TWA was assessed at HR below 125 bpm. Finally, the computation of TWA involves superimposing of the odd and even beats, as described in the last paragraph. Superimposed beats should be perfectly aligned within the QRS complex segment and only the ST segment may differ (see Figure 6). The alignment of QRS templates controls for level of noise and avoids false TWA detection. The leads that met the above evaluation criteria were selected for statistical analyses – the leads with maximum TWA at HR < 125, low noise, and perfectly aligned QRS complexes.

MTWA, Cambridge Heart Method. MTWA is a spectral method that uses 128 beats to create a spectrum (see Figure 5). Multiple spectra are generated and then averaged into one composite spectrum. TWA were measured in microvolt (μV) and defined as difference between alternans power, calculated at 0.5 cycles per beat frequency, and noise power, calculated at 0.44-.49 cycles per beat frequency.

In the present study, ICD patients had MTWA bicycle exercise test during which continuous ECG was recorded. The MTWA test was automatically interpreted within the CH2000; and the results were classified into “present,” “absent,” or “indeterminate” based on Cambridge heart criteria. The procedures for MTWA interpretation are described in detail in Bloomfield et al (2002). Briefly, microvolt T-wave alternans is defined as positive if there is sustained alternans (at least for 1 minute duration), with power ≥ 1.9 μV, and at heart rate < 110 beats per minute; MTWA test is defined as negative if the result does not meet criteria for positive TWA identification, if the maximum negative heart rate is ≥ 105, and if the noise level is ≤ 1.8 μV; and finally

MTWA test is classified as indeterminate if the result does not meet criteria either for positive or negative MTWA test.

In the present study the data on MTWA was collected during exercise and mental stress. Because of the MTWA Method's stringent criteria for positive test (e.g., sustained alternans for at least 1 minute at HR < 110), ICD patients in the current study did not meet the requirements for the positive test classification during mental stress challenge. Therefore for the T-wave alternans comparison of the two algorithms, MMA and microvolt, only data collected during exercise is used. In the present study MTWA was assessed through entire duration of the exercise challenge and through the recovery time after the challenge. Therefore, patients who had significant T-wave alternans during the challenge, but have not been classified as T-wave positive during exercise due to HR increase above the accepted requirement for this test, may been identified as TWA "positive" during recovery, when HR was lower.

*QTVI.* QT variability index is derived from digitized ECGs continuously monitored during rest, MS, EX and recovery. The detailed procedure for calculating QTVI is described in Berger (2003). Briefly, variance in QT intervals represents the magnitude of QT variability. This parameter is HR dependent. Therefore, in the calculation of QTVI, it is important to relate variability in QT to the variability in the heart rate. Series of QT and HR intervals are sampled at 4 Hz frequency. QT interval series resulting from ectopic beats and large abrupt deflections in the HR are eliminated. Linear trends found in the HR and QT interval series are removed by subtraction of the best-fit line. Then the HR mean (HRm), HR variance (HRv), QT interval mean (QTm), and QT interval variance

(QTv) are calculated from the respective time series. The normalized, log transformed, QT variability index is calculated by following formula:

$$QTVI = \log10 [(QTv/QTm^2) / (HRv/HRm^2)$$

In the present study, QTVI will be calculated during the 5-minute rest phases before exercise and mental stress. Assessment of QTVI during exercise does not allow making an accurate distinction between healthy individuals and patients at risk for future cardiac events, because at higher heart rates healthy individuals may also display high QTVI (Haigney, et al., 2009).

***Heart rate variability.*** HRV was derived from digitized ECGs continuously monitored during MS and EX. Spectral analyses of digitized R-R intervals were conducted. The spectral HRV analyses method has been previously described in Rottman et al. (1990). Spectral analyses yield periodic and aperiodic frequency components of HRV. Periodic frequency domains are HF (0.15—0.4 Hz) in $ms^2$ and LF (0.04—0.15 Hz) in $ms^2$. Aperiodic domains are ultra low frequency (ULF; $1.15 \times 10^{-5}$ to 0.00335 Hz) in $ms^2$ and very low frequency (VLF; 0.0033 to 0.04 Hz) in $ms^2$. Normalized units of HF during MS tasks and EX will be used in data analysis. HF is normalized (nHF) by dividing the absolute power of periodic spectral component (HF) by total HRV power, from which aperiodic components (ULF and VLF, e.g., < 0.01 Hz) are subtracted. This manipulation prevents very low frequency oscillations from masking other frequency components (HF and LF) (Aytemir, et al., 2000). In the present study, nHF will be calculated for entire duration of mental stress tasks and for entire duration of exercise.

***Ischemia.*** Dual-isotope single photon emission computed tomography (SPECT) was used to assess MS and EX induced ischemia. The detailed procedure is described in Kop et al (2004) and Akinboboye et al (2005). In brief, thalium-201 was injected during 15 minutes of the rest period. SPECT images of myocardial perfusion were obtained 10 minutes after isotope injection. The dose of the isotope was determined based on the participant's weight and ranged from 2.5 to 3.5 mCi. Two minutes into the anger recall task, 20 to 30 mCi of $^{99m}$Tc sestamibi was injected. Injection of sestamibi occurred at peak effort (85% of max HR). Patients continued to exercise for one minute after isotope injection. SPECT images were acquired at 45 minutes after MS and EX. A trained technologist (blinded to clinical information and type of stress) then analyzed the images using QPS software (Cedars-Sinai Medical Center) with a 20-segment, 5-point model of isotope uptake contrasting the myocardial perfusion of the region (0 = normal, 1 = mildly reduced uptake, 2 = moderately reduced uptake, 3 = severely reduced uptake, and 4 = no uptake). Summed scores at rest, MS and EX were calculated for the corresponding scans. In order to determine the severity of ischemia, differences between summed scores at rest and during challenges were calculated Additionally, summed difference scores between rest and MS or EX were used to determine if stress-induced ischemia was absent (0 to 3), or present (>3).

***Heart rate recovery.*** HRR recovery is typically assessed over a period of several minutes immediately after physical exertion ceases (Cole, Blackstone, Pashkow, Snader, & Lauer, 1999) and it is defined as a difference score: maximum heart rate during challenge (exercise or mental stress) minus HR at recovery after ending the challenge. In

a recent study done by Myers and colleagues (2007) the assessment of HRR at 2 minutes after exercise treadmill test in 1910 males was a stronger predictor of cardiovascular mortality, including SCD, during a 5 year follow-up, compared to clinical data on disease severity. In the current study, the post-MS and post-EX heart rate was assessed every two minute intervals starting at 30 seconds pos challenge. Therefore HRR in this study was measured based on standard procedures and was defined as a difference score: maximum heart rate (HR) during each task minus HR at 2.5 minutes post-MS and post-EX, respectively.

***Ejection fraction.*** Ejection fraction was determined by previous clinical catheterization (n = 24) or gated single-photon emission computed tomography (SPECT). In patients who had both, catheterization and gated SPECT, EF measures were correlated ($r = .75$, $p < .001$) (see (Akinboboye, Krantz et al. 2005)). The average time between previous angiography and participation in the TRIAD study was 31.9 months. Patients whose EF was determined through catheterization did not have intervening MI between cardiac catheterization and the TRIAD study.

In order to determine the EF using SPECT, Thalium-201 (2.5 to 3.5 mCi) was injected at rest and single-photon emission computed tomographic images were obtained after 10 minutes of the injection. Images taken at rest were reviewed by a trained technologist blinded to clinical information. Images were analyzed using QPS software (Cedars-Sinai Medical Center), using circular 180 acquisitions of 32 projections at 40 seconds per projection. Images were reconstructed into transaxial images using filtered backprojection with a ramp filter. After automatic reorientation, gated short-axis images

were processed using the quantative gated SPECT algorithm (see (Germano, et al., 1995)) and left ventricular end- diastolic and end-systolic volumes were calculated. The EF was calculated using the following formula:

[(end-diastolic counts — end-systolic counts) / (end-diastolic counts)] x 100

In the present study, EF was classified as low to moderate if it was ≤ 40% and as high if it was > 40%. Previous research indicated that EF ≤ 40% predicts future cardiac mortality and adverse cardiac events (Alidoosti, Salarifar et al. 2008). Furthermore, patients with EF ≤ 40% were found to be at higher risk for ventricular tachycardia and ventricular fibrillation than patients with EF > 40% (Gardiwal, Yu et al. 2008).

**Power Analyses**

In order to determine the number of subjects necessary to detect the proposed relationships, a pilot dataset from the TRIAD study was analyzed. Data were available for following variables collected during exercise: TWA, measured by Cambridge Heart Method, QTVI, EF, and HRR.

The primary goal of the current study is to investigate the multivariate hypothesis concerning the associations between non-invasive markers and TWA during exercise and mental stress challenges. Hence, power analysis was conducted for this multivariate hypothesis to estimate the sample size necessary to detect an effect. The multivariate effect of EF, HRR, and QTVI had a moderate size of $R^2 = .33$, $p = .005$ when predicting

TWA via the Cambridge Heart method during exercise. Using power of 80% and a two-tailed alpha level of .05, 27 subjects are needed to detect this moderate effect size.

The secondary goal of the study is to investigate the univariate associations of each marker to TWA during exercise and mental stress. Two-tailed independent sample t-tests were run on the pilot dataset using MTWA positive versus negative as an independent variable. QTVI, EF and HRR had large effect sizes, ranging from .74 to .95. Using power of 80% and a two-tailed alpha level of .05, 20 subjects per group are needed to detect this large effect size.

Based on the power analyses reported above, a sample size of 40 will be sufficient to detect the relationships hypothesized in the present study with 80% power.

**Statistical Analyses**

In order to investigate if levels of non-invasive markers of myocardial vulnerability and autonomic activity were different during exercise compared to mental stress challenges (Hypothesis I), Wilcoxon Signed Ranks Test (Ischemia), paired-sample t-tests (HF), and repeated analyses of variance (TWA and HRR) with time-varying covariates – covariates which values vary over time or over conditions within each participant – were performed. It should be noted that as a validity check for HRR, a series of preliminary paired t-test was performed to determine if there was a significant change from baseline to stress in HR during exercise and mental stress challenges. HRR was calculated by subtracting resting HR from HR at 2:30 post challenge (see the Methods for more details). In addition, since TWA and HRR during each challenge are heart rate dependent, a fair comparison of these variables across exercise and mental stress

challenges require controlling for differences in heart rate during each challenge. Prior to conducting the repeated ANCOVA, paired t-test was performed to compare resting HR, maxHR, and HR during max TWA between exercise and mental stress. If there was a significant difference between exercise and mental stress in max HR and HR during max TWA, max HR and HR during max TWA were included as covariates for HRR and TWA, respectively. Finally, in the repeated ANCOVA, TWA (or HRR) was included as the dependant variable with type of challenge (exercise versus mental stress) as the independent variable and HR during max TWA (or max HR) as the time-variant covariate.

For Hypothesis II (a), correlation analyses were conducted between MMA-TWA and QTVI, EF, HRR, Ischemia and HF in order to assess pairwise relationships among physiological variables during exercise and mental stress. In particular, Pearson's Product-Moment Correlation Coefficients were calculated when both variables are continuous in the correlation analysis; Spearman's Rank-Order Correlation Coefficients were calculated when both variables are categorical; and, Point-by-Serial Correlation Coefficients were computed when one of the variables in the pair is dichotomous and the other one is categorical.

For Hypothesis II (b), in order to compare the strength of the pairwise associations between MMA-TWA and QTVI, EF, HRR, Ischemia, and HR between exercise and mental stress challenge, a series of z-tests – tests which statistics have a standard normal distribution (i.e., z distribution) – was performed. One of the methods to compare two correlation coefficients (including Pearson's Moment Product, Spearman's Rank, and Coefficient of Determination (R-squared) in regression models) is to transform

the correlation coefficients such that each correlation coefficient has a standard normal distribution (Fisher & Bonynge, 1915). The two transformed correlation coefficients are then compared for statistically significant difference. The transformation used here is commonly known as Fisher's z transformation and the algorithm is: 0.5*ln[(1+r/1-r)]. Some researchers argued that correlation coefficients need to be transformed to t distribution instead of z distribution prior to testing; while others argue that z test (based on z distribution) is fairly robust for sample size greater than 10. In the present study, z test was chosen because the sample size is greater than 10. Specifically, the correlation coefficients between MMA-TWA and QTVI, EF, HRR, Ischemia, and HR were transformed during exercise and mental stress. z-tests were then performed to determine if the strength of the associations between non-invasive markers and MMA-TWA during exercise was different compared to during mental stress.

For Hypothesis II (c) and (d), linear regression analysis was conducted separately for mental stress and for exercise with MMA-TWA as the dependent variable and variables that were significantly correlated with MMA-TWA as the independent variables, using number of myocardial infarctions and use of beta blockers as covariates. To compare the collective effect of non-invasive markers on MMA-TWA during exercise versus during mental stress, the correlation of determination (R-squared) for each of the regression models was transformed via Fisher's transformation algorithm as mentioned above. The two transformed correlation coefficients were then compared in a z-test to determine whether the two were statistically significant different. To test Hypothesis II (c), the z score of the collective effect of the non-invasive markers to TWA was compared to the z score of each marker to TWA during both exercise and mental stress.

Further, to test Hypothesis II (d), the z score of the collective effect of the non-invasive markers to TWA during exercise was compared to that of the collective effect of the non-invasive markers to TWA during mental stress.

Finally, Chi-square tests were performed to compare the agreement of the two TWA assessment methods (MMA-TWA and MTWA) and to assess the relationship of MTWA to ischemia and EF.

Statistics were analyzed with SPSS release 16.0 for Windows (SPSS Inc., Chicago, IL). Significance levels were two-tailed and set at $\alpha = 0.05$ for all analyses. The alpha level was not adjusted for Type I error since the objective of the present study is exploratory in nature.

# Results

## Sample Characteristics

Fifty coronary artery disease patients with arrhythmic vulnerability and implantable cardioverter defibrillators (ICDs) met the inclusion criteria were included in the current study. Patients were treated with ICD implantation for the following reasons: 4 had sudden cardiac death (9.3%); 11 had syncope with ventricular fibrillation or tachycardia (25.6%); 7 had symptomatic ventricular fibrillation (16.3%); and 17 (39.5%) had other reasons for ICD therapy. Digital continuous ECG data of each patient was analyzed to determine the presence of TWA during exercise and mental stress. Participants were between the ages of 38-78 (Mean = 59, SD = 9.9). The sample consisted of 4 females (9.3%), 39 males (90.7%); 37 Caucasians (86.0%), 4 Black (9.3%), and 2 Hispanic (4.7%); 32 married (74.4%); 39 living with someone (90.7%); 23 employed (53.5%); and 42 had $\geq$ 12 years of education (92.7%). In terms of medical history which may influence current data analyses, 37 patients (86%) had previous myocardial infarction and 30 (69.8%) were on beta blockers during the study. Table 1 displays additional information about participants' medical history and health habits.

## Initial Data Examination and Transformation

Physiological data were examined for the quality of the recordings and were excluded from the analyses of hypotheses regarding mental stress or exercise: 1) if the quality of the ECG recordings were poor (n = 1 during exercise and mental stress); 2) when heart rate was above 120 beat per minute during exercise (n = 9) because at

HR>125 bpm healthy individuals may also have TWA (see the methods section); 3) if data points were abnormally high or low from expectation (i.e., two standard deviations above or mean on the variables of interest), including TWA, HRR, HF, and QTVI during exercise and mental stress (n = 6); 4) and if the patient was not able to engage in the exercise task (n = 1). The final sample size was 43 for the mental stress task and 33 for the exercise task. In order to have adequate sample size for the hypotheses, patients' records were included even if some of the physiological variables of interest were missing. As a result, number of records available was different for each hypothesis. See table 2 for the sample size available for each variable. The following explains the criteria and the procedures for data examination.

***T-wave alternans (TWA).***

*MMA-TWA (modified moving average method).* The highest TWA amplitude (maxTWA) measured in microvolts (μV) was selected for the analyses. TWA of the participants ranged from 2 to 12 μV (M = 6.5, SD = 2.3) during exercise and from 1 to 13 μV (M = 5.4, SD = 2.9) during mental stress. TWA-MMA during exercise had a moderate correlation with TWA-MMA during mental stress, $r_{pearson(p)} = 0.37$, $p = 0.034$ (See Figure 7). In contrast, Chi-square test between categorical MMA-TWA during exercise and categorical MMA-TWA during mental stress did not approach statistical significance.

According to the guidelines of "TWA physician's guide (2008-2009)," the HR during max TWA has to be below 125 beats per minute (bpm) in order to insure TWA specificity. That is, TWA is heart rate dependent (GE, 2009). In the current sample, HR

during maximum TWA ranged from 87 to 120 bpm during exercise and from 60 to 115 bpm during mental stress. Additionally, we tested the associations between TWA and HR during maxTWA for each challenge. maxTWA was not associated with HR during exercise, $r_p = -0.22$, $p = 0.21$, or during mental stress, $r_p = 0.07$, $p = 0.72$.

To ensure that the value of TWA is not an artifact, signal (TWA amplitude) to noise (noise amplitude) ratio was calculated for all TWA points during mental stress and exercise. This ratio was above 1.2 for all significant TWA data points during exercise and mental stress, see methods section on TWA assessment.

A categorical variable of MMA-TWA was created, in order to evaluate the agreement between two different methods of TWA assessment. MMA-TWA was dichotomized into positive (TWA $\geq 5$ μV) or negative TWA (TWA $< 5$ μV), based on Exner et al. (2007) study of post MI patients with arrhythmic vulnerability. This categorical TWA (MMA) data was used for chi-squared analyses. In contrast to the continuous MMA-TWA data, the agreement between categorical MMA-TWA during exercise and categorical MMA-TWA during mental stress did not reach statistical significance (see Table 3). As indicated in the two-by-two crosstabulation of categorical MMA-TWA during exercise versus mental stress, some of the cell counts were below 5, therefore Fisher's exact test was reported for statistical significance instead of the Pearson Chi-square statistic (Fisher's exact test: $p = .118$; Kappa Coefficient = .26, $p = .085$).

*MTWA (Cambridge Heart Method).* MTWA was collected during exercise only. Patients were categorized into positive, negative, and indeterminate based on Cambridge Heart criteria (Narayan, et al., 2006). 14 (34.1%) of the patients were positive, 9 (22.0%)

were negative, and 18 (43.9%) were "indeterminate." Previous research indicated that "indeterminate" TWA may be similar to positive TWA in predicting SCD or sustained VT (Bigger and Bloomfield 2007; Chow, Saghir et al. 2007). Therefore, in the current study, the "indeterminate" cases were combined with the positive cases. This dichotomized MTWA variable was used for categorical chi-squared analyses in order to compare MMA-TWA to MTWA.

***Heart rate recovery (HRR).*** HRR was calculated by subtracting the heart rate at 2.5 minute after cessation of exercise or mental stress from the maximum heart rate during exercise or mental stress. In addition, to ascertain the effect of exercise and mental stress procedures on HR increase, a series of paired sample t-tests were conducted to compare resting HR and max HR during each task. As expected, there was a significant increase in HR from rest to exercise, $t_{(40)} = 22$, $p < 0.001$, and also from rest to mental stress, $t_{(42)} = 9.5$, $p < 0.001$.

***Ejection fraction (EF), QT variability index (QTVI) and ischemia.***

*EF***.** For the purpose of the current study, values of resting ejection fraction were dichotomized into "low EF" (EF ≤ 40%) and "moderate-normal" (EF >40%); because patients with EF below 40% have higher likelihood of mortality in comparison to patients with moderate to normal EF (Bigger & Bloomfield, 2007). A total of 31 participants (81.6%) in the study were determined to have low EF.

*QTVI.* QTVI data were excluded if the values were abnormally high or low above the mean (i.e., 2 standard deviations above or below the mean). After the exclusion of patients with abnormal data (n = 2), resting QTVI ranged from -2.0 to 0.12 (M = -1.1, SD = 0.58).

*Ischemia.* In a present sample, 13 patients (41.9%) had evidence of ischemia on SPECT during exercise, and 11 (35.5%) had ischemia during mental stress. Prevalence of myocardial ischemia during exercise had moderate level of agreement with prevalence of ischemia during mental stress. See Table 4 for the 2-2 crosstabulation of ischemia during mental stress versus ischemia during exercise. Some of the cell counts were below 5, therefore Fisher's exact test was reported for statistical significance instead of the Pearson Chi-square statistic, Fisher's exact test: p = .021, Kappa Coefficient = 0.46, p = .01.

***High frequency (HF) of heart rate variability (HRV).*** HF of HRV data were not normally distributed. To achieve normality for testing the hypotheses, log transformation was applied to achieve normal distribution. After log transformation, HF ranged from -0.24 to 3.14 during exercise (M = 1.21, SD = 0.71), and from 0.35 to 3.33 during mental stress (M = 1.99, SD = 0.68). It is important to note that the HF data collected in the present study were not based on individuals with normal sinus rhythm and some other erratic rhythms (e.g., ventricular, supra-ventricular) may have been included in the analyses. See Malik et al. (1996) for further information on HRV measurement guidelines.

**Study Aim and Hypothesis I**

Study Aim I was to determine if there were significant differences between exercise and mental stress in TWA, HRR, Ischemia and HF. Repeated analyses of variance (ANOVA) with time-varying covariates, Wilcoxon Signed Ranks Test and paired-sample t-tests were performed where appropriate to investigate the following hypothesis.

***MMA-TWA.***

**Hypothesis I. It was expected that MMA-TWA during exercise would be higher in comparison to MMA-TWA during mental stress.** Repeated ANOVA was conducted to test these hypotheses. Given that TWA is heart dependant and that HR during max TWA was higher during exercise than during mental stress (Exercise HR = 104.2; Mental HR = 91.7, $t_{(32)} = 6.2$, $p < 0.001$), heart rate during each condition was controlled for (i.e., used as a time-varying covariate) while examining differences in MMA-TWA between exercise and mental stress. Results showed that prior to controlling for heart rate, there was a trend for TWA during exercise to be higher than TWA during mental stress, $F_{(1, 73)}=3.1$, $p = 0.08$. After controlling for heart rate during max TWA, TWA during exercise was significantly higher than TWA during mental stress, $F_{(1, 54.6)} = 11.8$, $p = 0.03$. However, the effect of HR during max TWA on MMA-TWA was not significant, $F_{(1, 68.8)} = 1.1$, $p = 0.29$, suggesting that MMA-TWA was not affected by HR. See Table 4a.

***Heart Rate Recovery (HRR).***

**Hypothesis I. It was expected that HRR during exercise would be faster in comparison to mental stress.** Repeated analysis of variance was conducted to test the difference between HRR during exercise and mental stress. Given that maximum heart rate was higher during exercise than during mental stress (Exercise HR =113.0; Mental HR = 78.3, $t_{(40)} = 13.7$, $p < 0.001$), maximum heart rate during each condition was controlled for when examining differences between HRR between mental stress and exercise. Prior to controlling for maximum heart rate, HRR was more rapid after exercise than after mental stress, $F_{(1, 53.5)} = 122.7$, $p < 0.001$. As expected, when maximum heart rate was controlled for, HRR after exercise was significantly faster than HRR after mental stress, $F_{(1, 73)} = 32.2$, $p < 0.001$. Also, there was a significant effect of maximum heart rate on HRR, $F_{(1, 54.7)} = 34.7$, $p < 0.001$. See Table 4b for results of repeated analysis of covariance.

***Myocardial Ischemia.***

**Hypothesis I. It was expected that during exercise patients would have higher prevalence of ischemia in comparison to mental stress.** This hypothesis was examined by Wilcoxon Signed Ranks non-parametric test. There were no significant differences in incidents of ischemia during exercise versus mental stress, $Z = -.71$, $p = 0.48$ (See Table 3)

***High Frequency (HF) of Heart Rate Variability (HRV).***

**Hypothesis I. It was expected that during exercise HF would be lower in comparison to mental stress.** This hypothesis was examined by paired t-test. As expected, HF was lower during exercise in comparison to mental stress, $t_{(28)} = -4.23$, $p < 0.001$ (See Table 2).

Additionally, we have examined the differences in systolic and diastolic blood pressure (SBP and DBP) reactivity between exercise and mental stress, calculated as the difference of scores between blood pressure during the challenge (exercise or mental stress) and resting blood pressure. In contrast to the TWA, HF and HRR, the blood pressure reactivity was higher during mental stress in comparison to exercise for SBP and DBP reactivity, $t_{(39)} = -2.2$, $p = .04$ and $t_{(39)} = -8.9$, $p < .001$, respectively.

**Study Aim and Hypotheses II**

***Study Aim and Hypothesis II (a).*** Aim II (a) was to assess the relationships between physiological variables during exercise and mental stress. Correlation analyses were conducted between MMA-TWA and QTVI, EF, HRR, Ischemia and HF in order to assess relationships among physiological variables during exercise and mental stress.

**Hypothesis II (a), relationships during exercise. It was predicted that during exercise resting QTVI and ischemia would be positively related to MMA-TWA, while EF, HRR and HF would be inversely related to MMA-TWA.** As was expected during exercise**,** patients with higher levels of TWA had higher levels of QTVI at rest ($r_p = 0.52$, $p = 0.004$, see Figure 8) and lower EF ($r_{spearman(sr)} = -0.38$, $p = 0.04$). See Figure 9.

These relationships remained after controlling for HR during maximum MMA-TWA, p = 0.002 and p=.056, respectively. There were no associations between TWA and HRR, Ischemia and HF. See Table 5a and Figures 10-12 for the results.

**Hypothesis II (a), relationships during mental stress. It was expected that during mental stress resting QTVI would be positively related to MMA-TWA, while EF, HRR and HF would be inversely, and ischemia would not be associated with MMA-TWA.** As was expected during mental stress**,** patients with higher levels of TWA had higher levels of QTVI ($r_p$=0.48, p = 0.002, See Figure 8) and lower EF ($r_{sr}$ = -0.43, p = 0.007). See Figure 10. These relationships remained after controlling for the HR during maximum MMA-TWA, p = 0.01 and p = 0.008, respectively. There were no associations between TWA and HRR, Ischemia and HF. See table 5b and Figures 10-12.

Additionally, relationships of QTVI with MMA-TWA were evaluated during exercise and mental stress. These associations were not statistically significant during exercise ($r_p$= 0.24, p = 0.26) and during mental stress ($r_p$=0.11, p = 0.54). We also evaluated relationships between MMA-TWA and severity of ischemia (represented by summed scores, see methods), during exercise and mental stress. The relationships between MMA-TWA and severity of ischemia, calculated as differences between summed scores at rest and during challenges, did not approach statistical significance during exercise ($r_p$= 0.13, p = 0.54) and during mental stress ($r_p$=0.15, p = 0.44). Furthermore, we investigated the relationships of resting HR with MMA-TWA during exercise and mental stress. Similar to the relationships of HRR and HF of HRV, these associations were not statistically significant during exercise ($r_p$= -0.07, p = 0.71) and during mental stress

($r_p$=-0.17, p = 0.29).

***Study Aim and Hypothesis II (b).*** Study Aim II (b) was to compare individual associations of EF, HF, HRR, resting QTVI and myocardial ischemia to TWA between exercise and mental stress.

**Hypothesis II (b). It was expected that individual associations between MMA-TWA and other markers of arrhythmic vulnerability would be stronger during exercise in comparison to mental stress.** To test the current hypothesis, z-test was conducted to compare the strengths of the associations between mental stress versus exercise (Fisher RA, 1915). In particular, the strength of the association between MMA-TWA and QTVI during mental stress was compared with the strength of the association during exercise. Likewise, the strength of the association between MMA-TWA and EF during mental stress was compared with the strength during exercise. Prior to conducting statistical tests, correlation coefficient statistics (including Pearson's Moment Product and Spearman's Rank) were standardized via Fisher's transformation (0.5*ln[(1+r/1-r)]). After the correlation coefficients were transformed into z scores, z test was performed to determine whether there was a statistically significant difference between the strengths of the associations during exercise versus mental stress. Results of the z-test showed that there was no significant difference in the strengths of the association between MMA-TWA and QTVI during mental stress task versus during exercise (Z = 0.16, p = 0.87), and also between MMA-TWA and EF during exercise versus mental stress (Z = 0.16, p = 0.87).

***Study Aim and Hypothesis II (c ).*** Study Aim II (c) was to assess and compare the collective effect of EF, HF, HRR, resting QTVI, and myocardial ischemia to TWA to individual effect of each marker alone to TWA.

**Hypothesis II (c). It was expected that the collective effect of the non-invasive markers to TWA would be stronger in comparison to relationships of each marker alone to TWA during both exercise and mental stress.** Linear regression analysis was conducted separately for mental stress and for exercise tasks with MMA-TWA as the dependent variable and variables significantly correlated with MMA-TWA (i.e., QTVI and EF) in the previous analyses as the independent variables. To compare the collective effect of the non-invasive markers on TWA with the individual effect of each non-invasive marker alone on TWA, Z tests were conducted to determine whether there would be significant differences between adjusted R-squared for the regression models and bivariate correlations. Specifically, square root of the adjusted R-squared from the regression models was transformed to z scores via Fisher's z transformation. The bivariate correlation coefficients were also transformed to z scores via Fisher's z transformation. The transformed adjusted R-squared was then compared with the transformed bivariate correlation coefficients via z test (see the data analysis section for more details).

*Relationships during exercise.* Linear regression analyses revealed significant relationship among TWA, QTVI and EF, adjusted $R^2 = 0.42$, $F_{(2,23)}=10.26$, $p = 0.001$.

This relationship remained significant after adjusting for potential confounding effect of the number of myocardial infarctions and beta-blocker use, $R^2$=0.43, $F_{(2,21)}$=9.6, p = 0.001 (See Table 6a) The coefficient of determination of the collective effect of QTVI and EF on TWA was larger ($R^2$ =0.43) than bivariate coefficients of QTVI on TWA (r = 0.27,) and also EF on TWA (r = 0.14). However, the results of z tests did not show that these differences were statistically significant (QTVI: Z = 0.76, p = 0.45; EF: Z = 1.27, p = 0.20).

*Relationships during mental stress.* Linear regression analyses revealed significant relationships among TWA, QTVI and EF, $R^2$ = 0.21, $F_{(2,30)}$ = 10.26, p = 0.01. This relationship remained significant after adjusting for the potential confounding effect of the number of myocardial infarctions and beta-blocker use, $R^2$ = 0.29, $F_{(2,27)}$ = 5.7, p = 0.01 (See Table 6b). Similar to the relationship among the physiological variables during exercise, the coefficient of determination of joint effect of QTVI and EF on TWA was larger ($R^2$ = 0.29) than bivariate coefficients of QTVI on TWA (r =0.23) and EF on TWA (r = 0.18). The results of z tests did not show that these differences were statistically significant (QTVI: Z = 0.26, p = 0.79; EF: Z = 0.57, p = 0.57).

Additionally we constructed regression models with all of the proposed noninvasive markers (e.g., HRR, HF, resting QTVI, EF, and myocardial ischemia) during exercise and mental stress. The collective effect of all the non-invasive markers on TWA was not statistically significant during exercise, $R^2$ = 0.33, $F_{(5,14)}$ = 2.3, p = 0.13, with QTVI being the only significant predictor (See Table 7a). The collective effect of all the non-invasive markers on TWA during mental stress was significant, $R^2$ = 0.36, $F_{(5,20)}$ = 3.2, p

= 0.03, with EF being the only significant predictor in the model. See Table 8b. Finally, we constructed regression models incorporating all of the proposed noninvasive markers during exercise and mental stress using step-forward method of entering the predictors. In the final model, only resting QTVI was selected by the computer as a significant predictor of MMA-TWA for both exercise and mental stress, $R^2 = 0.44$, $F_{(1,14)} = 10.0$, $p = 0.007$ and $R^2 = 0.26$, $F_{(1,20)} = 6.7$, $p = 0.02$, respectively.

***Study Aim and Hypothesis II (d).*** Aim II (d) was to compare the collective effect of noninvasive markers on TWA during exercise to the collective effect of these markers on TWA during mental stress.

**Hypotheses II (d). It was predicted that the joint effects of QTVI and EF to MMA-TWA would be stronger during exercise in comparison to mental stress.** z-test was conducted to compare the joint effect of QTVI and EF on MMA-TWA during exercise versus during mental stress. Square root of adjusted $R^2$ of regression models were standardized to z scores via Fisher's transformation. z-test was then performed on the z scores to determine whether there was a statistically significant difference between the joint effects of non-invasive markers on TWA. Results showed that there was no significant difference between the joint effect of QTVI and EF on TWA during mental stress versus during exercise task, $Z = 1.04$, $p = 0.30$.

**Additional Analyses for MTWA (Cambridge Heart method) during Exercise**

***Comparison of MMA-TWA versus MTWA methods. It was expected that MMA and the Cambridge heart method would have high level of agreement in identify patients on positive versus negative TWA.*** The agreement of the two TWA assessment methods for TWA during exercise was evaluated using Chi-Square test. The categorical MMA-TWA data during exercise was compared with the MTWA data during exercise. Prior to conducting the Chi-square tests, a 2-by-2 crosstabulation of MMA-TWA categorical data versus MTWA data was created to examine the cell counts of the agreed (i.e., positive versus positive, negative versus negative) and the disagreed (i.e., positive versus negative) categories (See Table 8). Some of the cell counts were below 5, therefore Fisher's exact test was reported for statistical significance instead of the Pearson Chi-square statistic. As predicted, the two methods have moderate level of agreement, Fisher's exact test: $p = .042$, Kappa Coefficient = 0.39, $p = .028$. In addition, Sensitivity and Specificity were calculated and indicated that the two methods appear to have adequate agreement. Sensitivity was calculated as: the number of cases that were both positive in MTWA and MMA-TWA/(the number of positive cases in MTWA). Specificity was calculated as: the number of cases were both negative in MMA-TWA and MTWA/(the number of negative cases in MTWA). Results showed that Sensitivity was 86%; that is, MMA-TWA also gave a positive results on 86% of the cases identified as positive by the Cambridge Heart Methods. Specificity was 50%; that is MMA-TWA gave a positive result on 50% of the cases identified as negative by the Cambridge Heart Method; in other words, the "false positive" rate was 50% by the MMA-TWA method.

***Relationships of MTWA with other markers (QTVI, EF, HRR, Ischemia and HF).*** Similar to relationships of MMA-TWA with physiological markers of arrhythmic vulnerability, MTWA significantly associated with QTVI at rest ($r_{sr}$ = 0.39, p = 0.02; see figure 13), and did not associate with HF during exercise and Ischemia during exercise (Fisher's exact p = .44, Kappa Coefficient = 0.18, p = .28). In contrast to relationships of MMA-TWA during exercise, MTWA significantly associated with HRR at 2.5 minutes ($r_{sr}$ = -.33, p = 0.04), (see figure 13) and had no association with ejection fraction (Fisher's exact p = .69, Kappa Coefficient = -0.045, p = .068). See table 10.

## Discussion

The primary aim of this study was to compare the magnitude and direction of the relationships among various non-invasive markers of malignant cardiac arrhythmias (QT variability index, QTVI; ejection fraction, EF; heart rate recovery, HRR; high frequency of heart rate variability, HF; and myocardial ischemia) to T-wave alternans (TWA) during exercise and mental stress. The results of the study showed relationships (1) between TWA and a marker of compromised myocardial substrate (EF), and (2) between TWA and a marker of myocardial vulnerability (QTVI). The present study did not find relationships (3) among markers of autonomic dysregulation (HF and HRR), and TWA, and (4) between other marker of myocardial vulnerability (myocardial ischemia) and TWA. The strength and direction of the relationships between non-invasive markers of malignant cardiac arrhythmias and TWA did not differ between exercise and mental stress. Individual associations of these markers to TWA did not statistically differ from additive effects of these markers on TWA.

**MMA-TWA assessment during exercise and mental stress**

To our knowledge our study is the first to assess MMA-TWA during exercise and mental stress using commercially available GE Marquette software. Considering the fact that MMA-TWA is a fairly new method and that existent literature on the studies of MMA-TWA is not consistent in terms of the cut-offs for the critical value for MMA-TWA as a risk stratifier for the arrhythmic events in this section we thought that it would be important in this section of the of the discussion to compare and contrast our findings to the results in the existent literature.

The TWA values in the present study during exercise (M = 6.5 μV) and during anger recall (M = 5.4 μV) were both lower in comparison to those reported in literature. For example, Kop et al. used a subsample of participants with ICDs (n = 23) from the TRIAD study and found that during exercise and anger recall ICD patients' TWA were 31.1 μV and 25.4 μV, respectively. The discrepancy may arise from individual differences in patient characteristics (the current study had a slightly bigger sample size); different versions of software used for the TWA assessment; and/or the guidelines used in TWA evaluation. Kop et al. (2004) used a non-commercial version of MMA-TWA; in contrast, the present study assessed MMA-TWA using software built into the GE Marquette Holter diagnostic computer system. It is possible that in order to avoid false identification of TWA in clinical practice, the commercial version of the software has a higher threshold for TWA detection and lower tolerance for noise in comparison to the non-commercial version used by Kop et al. Furthermore, the present study followed strict procedures of TWA evaluation using commercially available GE

Marquette software (see Methods) (GE, 2009). Based on these guidelines, the magnitude of noise had to be significantly lower than magnitude of TWA. Additionally, the TWA values are considered reliable only if the lead has low noise and prefect alignment of the QRS complexes during superimposing of the odd and even beats. In the current study, high values of TWA generally corresponded to relatively worse quality of ECG recordings and higher noise in comparison to ECG recordings of those with lower values of TWA. This may have lead to the exclusion of leads with higher TWA magnitude in the present study.

Other researchers in TWA research recognized the importance of setting forth standardized guidelines for evaluating TWA validity (Garcia Ede, 2008; Kumar, Kwaku, & Verrier, 2008; Verrier, Kumar, & Nearing, 2009). According to a recent publication of the "T-wave Alternans Physician's Guide: revision C" by General Electric (2008-2009; page 23), the values of MMA-TWA assessed during routine exercise testing are predictive of SCD if MMA-TWA is $\geq 65\mu V$ and the values of MMA-TWA are indicative of elevated risk for SCD if MMA-TWA is $\geq 20\mu V$-$30\mu V$. The new GE guidelines are based on TWA assessment of averaging factor of 1/8 beats, compared to the 1/32 beats in the present study. Averaging TWA factor every 8 beats allows faster tracking of TWA morphologies; however, the TWA values are also more susceptible to the effects of noise and may result in higher levels of TWA amplitude. TWA is a parameter which is partially dependent on beta-adrenergic receptor activation, and thus on chronotropic effects on the heart. Several studies have indicated that use of beta-blockers decreased incidence and magnitude of TWA occurrence (Klingenheben, Gronefeld et al. 2001; Rashba, Cooklin et al. 2002). In our sample, 68% of patients were on beta blockers during data collection,

which may resulted in attenuation of the TWA magnitude. It is important to note that the proposed cut-offs by GE Marquette were based on subject population from Finish Cardiovascular Study (FINCAVAS) were patients maintained their medication use during data collection. Finally, the lower values of MMA-TWA in the present study may have also been due to the low severity of CVD in the sample. During the 3-year follow-up in the TRIAD study, of the 50 ICD patients, less than 10 incidents of ICD discharge due to arrhythmic events (in 4 participants) were recorded, a very low incidence of arrhythmia. There were no deaths due to SCD in the TRIAD study during the follow-up period. The current sample may have been at lower risk of SCD compared to other samples reported in the literature and subsequently relate to lower levels of TWA during exercise testing.

**Hypothesis I**

Hypothesis I predicted that that compared to mental stress, during exercise patients would have (1) higher levels of TWA, (2) faster HRR, (3) higher prevalence of myocardial ischemia, and (4) lower HF level. This hypothesis was partially confirmed by the findings. Patients had higher levels of MMA-TWA, faster HRR, and lower levels of HF of HRV during exercise in comparison to mental stress. However, there were no differences in the incidents of myocardial ischemia between mental stress and exercise. TWA, HF, and HRR exercise versus mental stress. In the present study patients had higher levels of TWA, lower level of HF and slower HRR during exercise in comparison to mental stress. These findings possible are attributable to the differences in the magnitude and type of the sympathetic responses between two challenges. As was

reviewed in the Introduction, physical and mental challenge both produce an overall increase in the activity of the sympathetic nervous system via catecholamine and neural adrenergic receptors, and subsequent withdrawal in parasympathetic stimulation of the heart. However, exercise challenge is linked to higher levels of Norepinephrine (NE) in comparison to levels of epinephrine (Epi) and to predominant activation of beta-receptors (Dimsdale and Moss 1980; Goldberg, Becker et al. 1996; Winzer, Ring et al. 1999; Paavonen, Swan et al. 2001). In contrast, mental stress is linked to higher levels of Epi and either combined or predominantly alpha receptor activation (Dimsdale and Moss 1980; Goldberg, Becker et al. 1996; Winzer, Ring et al. 1999; Paavonen, Swan et al. 2001). T-wave alternans, heart rate variability (e.g., High Frequency) and heart rate recovery are parameters which are greatly affected by the heart rate (1996; Turitto, Caref et al. 2001; Narayan 2008). Cardiac responses, including heart rate, to epinephrine (Epi) and Norepinephrine (NE) are predominantly mediated by beta-adrenergic receptors (Billman, Castillo et al. 1997). Considering that exercise challenge leads to a higher activation of the beta receptors and thereby to a higher heart rates in comparison to mental stress it should have been expected that markers of arrhythmic vulnerability which are affected by the HR (TWA, HHR, and HF of HRV) would be more pronounced during activation of beta receptors; therefore, during exercise.

The published literature in the area of TWA assessment during mental stress is very sparse. To date, only two studies investigated MMA-TWA during mental stress (Kop, Krantz et al. 2004; Lampert, Shusterman et al. 2009); and, only one of these studies compared levels of TWA between mental stress and exercise (Kop, Krantz et al. 2004). Kop et al. found that levels of MMA-TWA during exercise were higher in comparison to

levels of MMA-TWA during anger recall. Moreover, and similar to the findings in the current study, MMA-TWA during mental stress occurred at lower HR compared to MMA-TWA during exercise.

***Myocardial ischemia exercise versus mental stress.*** The present study did not find significant differences in the presence/absence of ischemia between mental stress and exercise. Out of the 33 patients with valid ischemia data, 25 (76%) had ischemia during exercise, of which 15 (46%) also had ischemia during mental stress. Similarly to our study, other investigators did not find significant differences in ischemia occurrence during two challenges (Kop, Krantz et al. 2004; Holmes, Krantz et al. 2007). Moreover it has been suggested that 20-70% of patients who have exercise induced ischemia also have ischemia during mental stress (Krantz, et al., 1999). It is likely that occurrence of myocardial ischemia is not solely dependent on chronotropic effects of HR activation, and that other than beta-adrenergic pathways of ANS may be involved in the inducement of myocardial ischemia.

Additionally, it should be noted that 86% of patients in the current sample had a previous myocardial infarction, which may have resulted in permanent anatomic damage of myocardium and the presence of areas with low perfusion, independent of the magnitude of the ANS activation between two challenges.

**Hypotheses II (a, b, c, and d)**

This section discusses findings of Hypothesis II (a) and Hypothesis II (c), followed by findings of Hypothesis II (b) and Hypothesis II (d), which compared markers of sudden cardiac death between mental stress and exercise challenge.

***Hypothesis II (a).*** Hypothesis II (a) postulated that, for both exercise and mental stress challenge, ischemia and resting QTVI are positively related to MMA-TWA, while EF, HRR and HF would be inversely related to TWA. As predicted, for both challenges there was a positive relationship between MMA-TWA (marker of autonomic vulnerability) and resting QTVI (another marker of myocardial vulnerability) and an expected inverse relationship between MMA-TWA and EF (marker of compromised myocardial vulnerability). However, there was no relationship between TWA and markers of autonomic dysregulation (HF and HRR) during exercise or mental stress. There also was no relationship between TWA and ischemia during exercise or mental stress challenge.

*Relationship between resting QTVI and TWA.* In the present study, ICD patients with elevated QTVI at rest had high levels of MMA-TWA during both exercise and mental stress. These findings could be explained by the fact that both of these markers reflect the lability of repolarization processes in the heart. In a normal heart, the atrioventricular node is the only pathway for conducting impulses from the atrium to the ventricles (see Introduction section). When the heart is in a diseased state (e.g., fibrosis, scarring), variability of repolarization (recovery of the excitability of the myocardium) may allow the impulses to travel in a circlular pattern within the heart (re-entry), leading

to sustained malignant arrhythmias (see the Introduction Section). Since the T-wave on electrocardiogram represents ventricular repolarization, and the QT interval represents the time required for ventricular depolarization and repolarization, one would expect to find a relationship between these two indices of the heterogeneity of the action potentials in the heart.

Our findings confirmed previous research. In a study of 42 CAD patients with known arrhythmic vulnerability (Shusterman, et al., 2006), ambulatory ECG recordings were analyzed and revealed that both TWA and non-alternans variability in T wave amplitude increased before the onset of ventricular tachyarrhythmia. Haigney et al. (2009) investigated a sample of ICD patients (n = 47) from the TRIAD study, of which QTVI was highly correlated with continuous TWA during exercise, ($r = 0.54$, $p = 0.0004$).

In the present study of CAD patients with ICDs, markers of myocardial electrical instability TWA and resting QTVI had a positive relationship because they both assess variability of repolarization processes in the heart.

*Relationship between EF and TWA.* In the current sample, ICD patients with low ejection fraction (EF < 40) at rest had high levels of MMA-TWA during both exercise and mental stress. It is possible that in the present sample patients with reduced EF at rest had areas of damaged myocardium, which lead to the dispersion of the repolarization processes during challenges. Damage to the muscle of the heart (such as MI or cardiomyopathy) impairs the heart's ability to eject blood, and therefore reduces ejection fraction. In the current sample 86% of people had previous MI, which may lead to the

presence of damaged myocardium and subsequent reduction in the ability of the heart to pump blood. Additionally, these damaged regions (e.g. post-infarction fibrosis, or scar) may yield heterogeneous repolarization through interference with normal cell-to-cell coupling, which reduces the efficiency of electrical conduction and promoting action potential variability. This heterogeneity in the repolarization process is more evident when the demand on the heart increases, such as during exercise and mental stress (Narayan, 2008). Therefore, resting EF – a marker for compromised myocardium – is inversely associated with TWA under stress – a marker for repolarization variability in the heart.

This finding is in contrary to a study by Kop et al. (2004), which examined the relation between resting EF and TWA in a subsample of ICD patients from the TRIAD study. The inconsistency between these findings may arise from the differences in subject characteristics between the two studies, or from the lack of statistical power in Kop's study, which had a total sample size of 23 (the present study had 33 during exercise and 44 during mental stress).

Previous studies of TWA mostly focused on assessment of T-wave alternans's ability to predict future arrhythmic events at different levels of ejection fraction, but not on the relationship between the two. Overall, cardiac mortality drastically increases as left ventricular ejection fraction (LVEF) falls below 41% (Bigger & Bloomfield, 2007). The increase in cardiac mortality is mainly due to pump failure, but not arrhythmias, especially in patients with severely reduced EF (Narayan, 2008). These findings suggest that T-wave alternans predicts SCD (arrhythmic death) most accurately in patients with moderate reduction of EF (30-40%; (Gold, Bloomfield et al. 2000; Narayan, Smith et al.

2005)). Furthermore, most of the studies which found a link between malignant arrhythmias and TWA were done in patients with ejection fraction below 40%. In the current sample, 81% of the sample (n = 31) had EF < 40%, which is significantly lower than an ejection fraction of 55% or above in a healthy individual (Bigger & Bloomfield, 2007).

*Relationship between Myocardial Ischemia and TWA.* Contrary to the hypotheses, there was no association between MMA-TWA and myocardial ischemia, a marker for myocardial vulnerability, during exercise or mental stress. Previous research on the relationship between myocardial ischemia and TWA has yielded mixed findings. Consistent with the current findings, Kop et al. (2004) did not find an association between MMA-TWA and myocardial ischemia during exercise or mental stress in subsample of the ICD patients from the TRIAD study. Also, Kovach and Nearing (2001) found that induction of anger in canines resulted in TWA increases with or without myocardial ischemia.

However, other studies have shown that TWA may be associated with myocardial ischemia (Nearing, Oesterle et al. 1994; Verrier, Kumar et al. 2009). For example, presence of ischemia is linked to the exaggeration of spatial dispersion of the repolarization in myocardium in animals (Hashimoto and Suzuki, 1984). Furthermore, ischemia leads to amplification of TWA in a human study (Narayan & Smith, 1999). Nearing and Oesterle (1994) studied both humans and dogs and reported TWA increases and subsequent ventricular fibrillation, as a result of myocardial ischemia (heart rate was held constant).

It should be noted that the lack of association between TWA and ischemia may also be attributed to some limitations in the current study. First, a majority of the sample was on beta-blockers, it has been reported that beta-adrenegic blockade reduces amplitude and presence of TWA (Narayan 2008; Verrier, Kumar et al. 2009). Even though ischemia may exaggerate the spatial dispersion of repolarization, especially during challenges, use of beta-blocking agents decrease these occurrences, which in turn leads to low levels of MMA-TWA, creating a floor effect for the association between TWA and ischemia. Secondly, the assessment of myocardial ischemia in the present study was conducted by the SPECT technique, which is known to be susceptible to human errors during imaging and image evaluation (see Gordon and Barry, 1994 for more details). Specifically, Eiserner and Churchwell (1988) reported that patient's physical motion during SPECT imaging may result in images that imitate perfusion defects on the heart scans, even with a movement that is only 3 mm (Eisner, et al., 1988). False identification of ischemia with the SPECT images has also been linked to presence of left bundle brunch block, left ventricular hypertrophy, overweight, and a number of other factors (Gordon, Barry et al. 1994). The current findings are as likely to be subject to the SPECT image artifacts as the other studies.

*Relationships between Markers of Autonomic Dysregulation (HF of HRV and HRR) and MMA-TWA.* Contrary to the predictions, there were no associations between MMA-TWA and markers of autonomic vulnerability, high frequency of HRV, and heart rate recovery during exercise or mental stress. Heart rate recovery and high frequency of heart rate variability are proposed markers that reflect vagal modulation on the heart.

Previous research has linked changes in autonomic nervous system activity (such as increased sympathetic activity and/or decreased vagal stimulation) to increases in TWA occurrences and TWA magnitude (Verrier, et al., 2009). The inconsistency between the present results and previous research may be attributed to choice of measurements of the autonomic dysregulatation, beta-blocker use in the sample, and other methodological issues during data collection and ECG analyses.

In the present study, HF of HRV was collected during the first stage of exercise and during anger recall. It is likely that the decrease in high frequency during the challenges was simply due to normal physiological response to an increased demand on the cardiac muscle, and does not necessarily reflect pathological imbalances in autonomic regulation of the heart. Furthermore, the current HF of HRV data was collected from ICD patients, and some other erratic rhythms (e.g., ventricular, supra-ventricular) may have been included in the analyses. This would lead to incorrect estimation of the HF of HRV; in particular in the present data the values of HF for some patients were overestimated. This particular issue, the presence of erratic rhythms during HF calculation, in the current HF data was ascertained by Dr. Phyllis Stein at the HRV lab of Washington University School of Medicine, one of the developers of Task Force guidelines for HRV assessment (1996). See Malik et al. (1996) for further information on HRV measurement guidelines.

In addition, the use of beta blockers in the current ICD sample may have resulted in inaccurate representation of the state of the autonomic regulation on the heart. In a study of 700 cardiac patients who were on beta-blockers, Huikuri and colleagues (2003) assessed various ECG based arrhythmic risk variables (HRV, baroreflex sensitivity, QT dispersion and etc.) during an average of 43 months follow-up, there were 22 cases of

SCD but neither of the autonomic markers, HRV and baroreflex sensitivity, and other ECG based parameters were predictive of future malignant arrhythmic events. The authors concluded that prognostic/diagnostic value of the autonomic markers in patients using beta blockers may be weakened.

The link between HF and cardiac arrhythmias is not yet fully established in humans (Kuss, Schumann, Kluttig, Greiser, & Haerting, 2008). For example, Exner et al. (2007) and Atiga et al. (1998) found that MMA-TWA and resting QTVI, rather than heart rate variability, were predictive of future arrhythmic events in CAD patients. In contrast, Odemuyiwa et al. (1993) and Pozzati et al. (1996) found that decreased HRV was predictive of life threatening arrhythmias in cardiac patients. Further, in large-scale studies of post-MI patients, such as European Myocardial Infarction Amiodarone Trial (EMIAT, N =743) and Autonomic Tone and Reflexes after Myocardial Infarction (ATRAMI, N = 1139), HRV was found to be predictive of future cardiac death but not of future arrhythmic events (Lloyd-Jones, Martin, Larson, & Levy, 1998). Perhaps, reduced HRV is prognostic of overall cardiac mortality, but not mortality specifically caused by arrhythmias.

As in the case of HRV, the relationship between HRR and TWA has not yet been directly examined; only a few studies found a relationship between HRR and arrhythmias in animals (Smith, et al., 2005) and in humans (Jouven, Empana et al. 2005; Guazzi, Myers et al. 2009). Jouven and colleagues (2005) followed up 5713 healthy men for 23 years and found that HRR profiles were predictive of SCD episodes. In a recent study of heart failure patients by Guazzi et al. (2009), heart rate recovery below 17 bpm was associated with increased risk of SCD and death due to a pump failure. However, in

contrast to these investigations, the Finnish Cardiovascular Study (FINCAVAS) was not able to predict SCD (HR=1.28, CI=0.59-2.81) during 47 months follow-up of 2029 cardiac patients based on diminished HRR after exercise testing (Nieminen, Leino et al. 2008). Nonetheless, patients who had HRR < 18 bpm were at high risk of overall cardiovascular mortality during follow-up (HR= 2.39, CI=1.34-4.26). Like diminished HRV, decreased HRR is associated with increased mortality (Cole, Foody et al. 2000; Nishime, Cole et al. 2000; Lipinski, Vetrovec et al. 2005; Guazzi, Myers et al. 2009). For example, Cole et al. (2000) investigated 5234 healthy individuals. A heart rate recovery of < 42 bpm at 2 minutes after exercise was associated with 2.58 increased risk for mortality from all-causes during 12 years of follow up, even after controlling for resting HR, resting blood pressure, smoking, and other associated factors. Perhaps, and similar to diminished HRV, HRR is a more accurate predictor of overall cardiac mortality, than arrhythmic death.

In sum, the null findings of the relationship between MMA-TWA and markers of autonomic deregulation on the heart (HRR and HF) may be due to quality of the data and beta-blockers. In addition, based on previous findings, HRR and HF may not measure the same physiological phenomena in patients as TWA. HRR and HF may be more predictive of overall cardiac mortality, while TWA is predictive of arrhythmic vulnerability.

***Hypothesis II (c).*** Contrary to our hypothesis, the collective effect of the non-invasive markers to TWA was not stronger in comparison to relationships of each marker

alone to TWA during both exercise and mental stress. Note that despite the lack of statistical significance, the R-squared coefficient of the collective effect of resting QTVI and EF to MMA-TWA appeared to be higher ($R^2$ =.43 for the exercise; and =.29 for mental stress) in comparison to individual associations of these markers to MMA-TWA ($r^2$=.14-.27). There are several plausible explanations for the present results. T-wave alternans and QTVI assess the lability of the repolarization processes. Diminished EF is a marker for existent myocardial damage, which may lead to dispersion of depolarization and subsequent dispersion in repolarization. Both QTVI and EF were measured at rest in the current study, and they may reflect the same phenomenon -- preexisting repolarization lability due to myocardial damage. Consequently, they account for the preexisting part of the repolarization inhomogeneity in TWA during exercise and mental stress. In support of this rationale for the findings, the correlation between QTVI and EF was weak to moderate strength of 0.23. Additionally the lack of differences between individual associations of the markers to TWA and their collective effect on the TWA may have been due to the small sample size for the regression analyses. Based on the power analyses for this study, the sample size for the regression models should have been 40. However, due to the quality of the data on the TWA and QTVI and the fact that not all participants had information on their resting EF, the sample size for the regression models was reduced to 25 patients for the exercise, and to 32 patients for mental stress.

It is important to note that this study did not assess the collective ability of the markers to predict malignant arrhythmias. The objective of the current investigation was to assess the relationships of these indices (e.g, EF and QTVI) to TWA. Therefore, these findings do not necessarily suggest that collective assessment of noninvasive markers of

arrhythmic vulnerability does not improve the risk stratification of patients at danger for SCD and malignant arrhythmias. For example, Exner and colleagues (2007) were able to identify patients at risk of SCD or cardiac arrest using a combination of risk stratifiers (TWA, EF, and impaired HRT) with higher accuracy than using TWA alone. The complex interplay of myocardial vulnerability (QTVI and ischemia), damaged myocardial substrate (EF), and autonomic dysregulation (HRR and HF) are required for the occurrence of malignant arrhythmias (Zareba & Moss, 2003). The collective effect of the non-invasive markers on identification of the individuals at higher risk for SCD warrants further investigation.

***Hypothesis II (b and d).*** Contrary to our hypotheses, relationships of non-invasive markers to MMA-TWA were not stronger during exercise in comparison to mental stress. These null findings may be attributed to 1) the time period that measures were assessed, 2) the severity of arrhythmic disease in this sample, 3) beta-blockers use in the sample or 4) to lack of a relationship between the type of challenge and the magnitude of the association of the markers to TWA. QTVI and EF were both measured at rest prior to each challenge, while MMA-TWA was measured during exercise and mental stress. Moreover, MMA-TWA was assessed during stage one of the exercise because of the high noise of the ECG recordings, which is a subliminal level of physical exertion. Also, over 80% of the participants were on beta-blockers, which may have blunted their sympathetic response to exercise. Additionally, the current sample did not have any reports of malignant arrhythmias during 3 years of the follow-up. Therefore, the

arrhythmogenic markers may have been in their “normal/healthy” range regardless of the type of the stressor used.

There is a growing body of research indicating that the activation of the autonomic nervous system and cardiovascular responses may differ between exercise and mental stress (see Introduction for more details). For example, ischemia and TWA tend to occur at lower HR during mental stress challenge in comparison to exercise (Krantz, Santiago et al. 1999; Kop, Krantz et al. 2004). During exercise, there is a prominent increase in norepinephrine, whereas during mental stress there is a pronounced increase in epinephrine. When there is a demand on the heart muscle, whether caused by mental stress or exercise, the activation of sympathetic nervous system follows and subsequently leads to increases in HR, contractility and cardiac output. These increases are more pronounced during exercise, especially with heart rate. The lack of differences in the magnitude and direction of the relationships of arrhythmic vulnerability indices between exercise and mental stress in the present study may indicate that psychological stress in comparison to physical challenge involves different pathophysiological pathways in increasing the arrhythmogenic activity, which may not be solely explained by the magnitude of beta-adrenergic system activation.

**Relationship between MTWA (Cambridge heart method) and other markers**

Currently microvolt T-wave (MTWA) is a leading non-invasive marker used to identify patients at risk for future cardiac arrhythmias (Narayan, 2008). MTWA data was available for a subsample of patients (n = 32) during exercise in the current study. We found moderate agreement between MTWA and MMA-TWA (Kappa = .39). In

particular, MMA-TWA had high sensitivity when was compared to microvolt -TWA (86%). The specificity of MMA-TWA was moderate (50%): MMA-TWA identified 5 out of 10 people identified by MTWA as being negative on the TWA testing. These findings were similar to prior research comparing these two methods (Cox, Patel et al. 2007; Selvaraj and Chauhan 2009). For example, Chauhan and colleagues compared the two methods using ambulatory ECG and synthetic ECG recordings. Similar to the findings in the current study, they found that MMA-TWA method tends to overestimate the TWA in comparison to microvolt-TWA assessment in both ambulatory and synthetic ECGs. In a clinical trial of 41 CAD patients, Cox and colleagues (2007) compared MMA and microvolt TWA methods during simultaneous ventricular and atrial pacing at 109 beats per minute. They also found higher levels of "positive" TWA detection (higher future risk for SCD) with MMA, as compared to the MTWA method, in predicting cardiac events (Cox, et al., 2007).

We also investigated the relationship of MTWA to other markers of arrhythmic vulnerability. Similar to the findings with MMA-TWA, MTWA was associated with resting QTVI but not associated with HRR after exercise. However, in contrast to the MMA-TWA findings, MTWA was not associated with resting EF prior to exercise challenge. Differences in findings between MMA-TWA and MTWA may be explained by small sample size and methodological differences between these two methods of T-wave evaluation. Specifically, since MTWA is a dichotomized variable, statistical power needs to be greater in detecting a statistically significant relationship between MTWA and EF (which is also a dichotomized variable) compared to power in detecting a relationship between MMA-TWA (which is a continuous variable) and EF. Note that 19

out of 23 patients identified by MTWA as positive had low resting ejection fraction; that is, MTWA had high specificity at identifying patients at high risk was (82.6%). However, MTWA had low sensitivity (23%), identifying 10 out of 13 patients as being “negative” (lower risk) had low EF at rest (higher risk).

In contrast to MMA-TWA findings, MTWA was significantly associated with HRR, so people with diminished HRR after exercise were more likely to be identified as MTWA positive during exercise testing. The dissimilarity in the relationship with HRR between two methods may have come from the procedural differences in TWA assessment. As was stated in the Methods section, MMA-TWA was assessed only during the first stage of the exercise, which is a subliminal level of physical exertion. On the other hand, MTWA was diagnosed as positive if the patient had met the criteria (see the Methods) for the positive T-wave alternans during entire exercise test and part of the recovery. Therefore, MTWA is expected to be more accurate in capturing sympathetic activation during exercise in comparison to MMA-TWA. Heart rate recovery reflects induced vagal tone on the heart, and its magnitude depends on the extent of prior sympathetic system activity. It is possible that MTWA and HRR are better indicators of the overall sympatho-vagal tone of ANS during exercise and the recovery time in comparison to MMA-TWA.

**Study Limitations**

The significance of these findings are decreased by the small sample size, cardiovascular medication usage (which may affected the correct evaluation of the sympato-vagal tone on the heart in this patient population), and the absence of a control

group (which limits the construct and external validity of the findings). In addition to the absence of a control group, we used T-wave alternans as a proxy for sudden cardiac death and malignant arrhythmias, which further limits the construct validity and clinical significance of the present results.

***Statistical power.*** One of the major limitations of this study was the small sample size for hypotheses testing (N = 33-44). Post hoc power analysis revealed that adequate power was obtained for the Hypothesis I. However, the present study was underpowered for the analyses of Hypotheses II (a) and (b). The effect sizes of the pairwise comparisons in the Hypotheses II (a) ranged from .38-.53 with power level of 50%-73%. In order to detect these relationships with moderate effect size and power of 80% a sample size of n=52 is needed. Additionally, the alpha level for the pairwise comparisons in the Hypothesis II (a) was not adjusted to the number of comparisons, which means that a Type I error is possible. Further, the sample size was slightly insufficiently powered for the regression analyses of Hypothesis II (b); the effect size were $R^2$=.38 and $R^2$=.52 with power of 74% and 76% in sample sizes of 33 and 25 for mental stress and exercise, respectively. Finally, the post hoc analyses were not sufficiently powered. This is mainly due to the fact that these analyses were not considered in sample size estimation.

Based on the effect sizes of the present findings and post hoc power analyses, and in order to detect relationships in proposed hypotheses with moderate effect size and a power of 80% at an alpha level of .05, a sample size of 52 is required.

***Cardiovascular medication use.*** Another limitation was that the majority of the sample

was taking beta-blockers during the study. Considering that this group of drugs acts as antagonists of sympathetic nervous system, the relationships between the markers of autonomic vulnerability (HF of HRV and HRR) assessed during exercise and mental stress may have been inaccurate due to blunted sympathetic activation during the challenges. Additionally, this may lead to lack of the differences in the relationships of the markers with TWA between exercise and mental stress, because the magnitude of the sympathetic activation did not considerably differ between the challenges.

***Timing and accuracy of the data assessment.*** Additionally, there were some concerns in timing and accuracy of the laboratory assessment. The values of heart variability were based on not only normal heart beats, but also some of the erratic beats, which may lead to incorrect representation of vagal tone on the heart during challenges. Further, because of the low quality of ECG recordings, the TWA-MMA was estimated only during first stage of the exercise, which is a low level of physical exertion. This may have contributed to the lack of differences in the comparisons of the relationships between exercise and mental stress. Finally, all of the variables were collected in a laboratory setting which may have limited the generalizability of the findings. It is possible that assessment of the ECG parameters over the period of time (e.g. 24 hour Holter) would allow for a better estimation of the arrhythmic vulnerability in this sample.

***Absence of control group.*** The current study did not include a control group and thus limits the external validity of these findings. Absence of the control group limits our ability to conclude that proposed indices of arrhythmic susceptibility are reflecting the

diseased processes of the heart and actually are valid in differentiation of individuals at high and low risk. It is worth noting that, prior studies that have examined subsamples of the TRIAD study (Kop, Krantz et al. 2004; Haigney, Zareba et al. 2009), healthy controls had consistently low levels of TWA and QTVI in comparison to ICD patients (used in the present study). Moreover, the relationships among markers of arrhythmic vulnerability (TWA and QTVI) were statistically significant only in ICD patients (Kop, Krantz et al. 2004; Haigney, Zareba et al. 2009).

***Absence of hard end-points measure.*** Lastly, there were no hard outcomes in this present research, such as malignant arrhythmias, SCD, and cardiac arrest. This limits the clinical significance of the findings. We were unable to evaluate the predictive power of TWA, QTVI, EF, HRR, HF and myocardial ischemia of arrhythmic events. Moreover, we are unable to estimate if usage of a combination of markers would be beneficial in risk stratification of patients for SCD. Finally, the question of whether assessment of these parameters during mental stress has any diagnostic clinical value cannot be answered fully with the present findings.

***MMA-TWA software limitations.*** Lastly, the use of MMA-TWA algorithm within Holter GE Marquette is a very labor intensive process which requires well trained technicians to ensure the accurate evaluation of TWA. Moreover, the minimal ECG data length required by the software is restrictive. T-wave alternans estimation is based only upon normal heart beats or beats generated by sinoatrial node. Therefore, it is crucial that all heart beats on the continuous ECG are properly identified as normal, supra-ventricular

or ventricular. GE Marquette software tends to mislabel heart beats, which requires labor-intensive manual correction. Furthermore, the MMA-TWA algorithm within GE Marquette has very a strict requirement on the time length of the ECG recordings analyzed: they have to equal or be longer than 5 minutes. The MMA-TWA method tends to present great variability in TWA values across the ECG leads within the same recording and tends to overestimate the TWA in noisy leads. Therefore, the evaluation of MMA-TWA using this commercially available diagnostic tool requires a well-trained operator to ensure accurate T-wave estimation.

Taken all together these concerns with MMA-TWA use may significantly limit the diagnostic utility of this tool, since it requires substantial amount of man-hours and specially trained ECG technician to correctly evaluate TWA and avoid artifact detection.

**Study Implications**

This study further adds to the body of research on non-invasive markers of cardiac arrhythmias, exploring the contributing factors for formation of the arrhythmias, and various methods of non-invasive assessment of the risk for sudden cardiac death. Sudden cardiac death (SCD) accounts for almost half of CHD deaths (C. S. Fox, et al., 2004). ICD implantation currently is the primary and sole prevention strategy for this disease (Filion, Xie et al. 2009). ICD implantation is a costly and invasive procedure. It is also difficult to identify individuals at risk on time, since death often is the only and last symptom of this disease. There is an increasing demand for the development of risk stratification algorithms to accurately categorize people that are at risk for cardiac arrhythmias, and people that would significantly benefit from ICD implantation. This

study was unique, because it evaluated the relationships between various possible markers of arrhythmic vulnerability during two types of stressors known to elicit different autonomic and cardiovascular responses in healthy individuals and patients.

***Relationships of TWA with other markers of arrhythmic vulnerability.*** In the present study we found moderate associations of QTVI and EF to TWA and no associations of markers of autonomic dysregulation to TWA. Both high QTVI and diminished EF have been linked to future malignant arrhythmias (Atiga, Calkins et al. 1998; Haigney, Zareba et al. 2004; Piccirillo, Magri et al. 2007). The present data suggests that QTVI, EF, and TWA may be independent risk indicators for arrhythmic vulnerability. We examined the interrelationships among QTVI, EF and TWA to demonstrate the extent to which these indices assess the same phenomena, such as repolarization variability and subsequent arrhythmic vulnerability. The moderate correlation of QTVI and EF with TWA implies that these indices are not duplicates and may make unique contribution in the SCD risk stratification of patients. This further underscores the importance of composite vulnerability index assessment. Moreover, the ability of TWA to predict SCD is diminished in patients who have widened QRS complexes on ECG, which is very common for the heart failure patients (Narayan, 2008). QTVI on the other hand is not affected by this abnormality in QRS. Additionally, QTVI can be measured at rest and may serve as a substitute risk stratifier in patients who cannot exercise and in patients who have ECG abnormalities.

The lack of relationships with the markers of compromised vagal tone on the heart (high frequency and heart rate recovery) and TWA in ICD patients on beta-blockers

may suggest that assessment of markers of the autonomic tone is not clinically prognostic in this population. It is possible that the use of beta-blockers affects the clinical importance of the relationships in these indices. This is further confirmed by several previous investigations. Rashba et al (2002) demonstrated that selective blockade of vagal nerve did not impact TWA during atrial pacing in humans. It is important to note that atrial pacing increases the heart contractility without sympathetic influence, therefore it is an artificial environment, which distorts the overall picture of the autonomic tone on the heart. Beta blockers create similar situation, they diminish sympathetic influences of autonomic nervous system by acting as antagonist of adrenergic receptors in the heart. Huikuri and Tapanainen (2003) demonstrated that the use of beta-blockers leads to diminished capacity of commonly used ECG markers to identify patients at risk for future cardiac events. This conclusion is further supported by the fact that the only significant relationships found in this study between TWA and other markers were with arrhythmic indices assessed at rest (QTVI and EF). This may suggest that, in order to increase the accuracy of risk stratification in patients who use beta blockers, the interpretation of markers of autonomic regulation should be approached with caution. Moreover, in this group of patients, assessment of the markers of myocardial vulnerability (TWA and QTVI) may have better clinical prognostic value if using information collected from ambulatory ECG, in comparison to using information collected during exercise-testing, since there a lesser dependency of the measures on sympathetic system activation during ambulatory ECG.

***Relationships between exercise and mental stress.*** Our study demonstrated that even after controlling for HR, there remained a different TWA magnitude between exercise and mental stress. This may suggest that chronotropic effect of sympathetic nervous system activation is not the only contributing factors to cardiac arrhythmias. This may further underscore the importance of SCD prevention; since most commonly used anti-arrhythmic drugs (e.g. beta-blockers) are predominantly seeking the reduction of chronotropic effects, which may not be enough to stop a malignant arrhythmia in a patient. For example, several studies demonstrated that although the use of beta blockers leads to decreases in the magnitude and occurrence of TWA, beta blockers do not completely suppress the presence of lability of repolarization processes (Verrier, et al., 2009). Lastly, that this finding and incidents of ischemia did not differ between two stressors may further confirm that there are dissimilarities in cardiovascular and nervous system responses between exercise and mental stress which cannot simply be attributed to differences in the magnitude of the sympathetic system activation.

We did not find any differences in the magnitude of the association of the markers of arrhythmic vulnerability to TWA between exercise and mental stress. According to Zareba and Moss (2003), several factors have to come into play in order for a malignant arrhythmia to occur. Our results demonstrate that, even though TWA magnitude was higher during exercise in comparison to mental stress, the combined associations of EF and QTVI to TWA did not differ between two stressors. That is, the complex vulnerability level required for increased arrhythmic activity did not differ between the two challenges. These findings may highlight the ability of mental stress to trigger adverse cardiac events, even at lower heart rates. Furthermore, these results may suggest

that in comparison to exercise, pathophysiological mechanisms leading to mental stress induced arrhythmias may have different autonomic nervous system pathways (Kop, Krantz et al. 2004). Previous research has demonstrated the ability of laboratory anger recall test to induce an increase in TWA and trigger persistent malignant arrhythmias requiring several shocks for the termination (Lampert, et al., 2000; Lampert, et al., 2009). Moreover, TWA assessment is predictive of future arrhythmias in the ICD patients during mental stress (Lampert, Shusterman et al. 2009). Taken together, for patients who cannot perform diagnostic exercise-TWA-testing due to the severity of cardiac disease, mental stress may be a possible substitute. In today's society, stress is more likely to be psychological, not physical (Glass & Singer, 1972). Moreover, in contrast to exercise, psychological stress does not have clear beginning or end (Krantz & McCeney, 2002; McEwen, 2004) and cannot be simple "terminated". These further, emphasize the importance of the studies which explore the autonomic and cardiovascular mechanisms by means of mental stress adversely impacts physical health.

***Prognostic value of MMA-TWA assessments.*** The assessment of T-wave alternans via moving average analyses method is a fairly new technique, and has only recently been accepted by FDA as one of the diagnostic methods for identification of patients at risk for SCD. To date, there are only a few studies that compared the MMA algorithm to the more widely accepted microvolt T-wave assessment method (Cox, Patel et al. 2007; Selvaraj and Chauhan 2009). Therefore, the present study contributes to growing body of research on the value of MMA-TWA as a clinical marker of future arrhythmic vulnerability. Previously, Cox et al. (2007) used atrial passing in order to

induce a TWA increase; and, Selvaraj et al. (2009) assessed TWA in ambulatory and synthetic ECGs. Neither of these two studies used the commercial versions of the software for the MTWA and MMA-TWA analyses currently offered to physicians. To our knowledge, our study is the first to use commercial diagnostic software for MMA-TWA (GE Healthcare), as well as for microvolt-TWA (Cambridge Heart) for TWA analyses. To our knowledge, our study is also the first to investigate relationships of the two during exercise and mental stress tastings. As with prior investigations, we found that the MMA-TWA method, in comparison to MTWA, lacks in specificity and tends to misclassify low risk patients as being at high risk for arrhythmias. Therefore, based on previous and current findings, the results of TWA assessment via MMA algorithm should be interpreted with caution in the identifying "positive" TWA.

***Future directions.*** An unanswered question from the present study is whether these non-invasive indices of arrhythmic vulnerability (TWA, QTVI, EF, HRR, myocardial ischemia, and HRV) have any predictive value for the early identification of the individuals at risk of SCD, cardiac arrest, or malignant arrhythmias. In the future, we would also like to determine if using the composite measure for the risk stratification is superior to using individual markers. Additionally, it would be important to evaluate the impact of cardiovascular medication on the predictive power of the non-invasive markers of SCD. In order to answer these scientific questions and further investigate the differences in pathophysiological pathways of central and autonomic nervous system between mental stress and exercise induced arrhythmias a prospective follow-up study

consisting of three phases (laboratory, ambulatory ECG monitoring , and a prospective follow-up) of patients with known arrhythmic vulnerability (e.g. ICD) is warranted.

In summary, MMA-TWA was significantly higher during exercise in comparison to mental stress regardless of the HR, suggesting that central and autonomic nervous system pathways leading to heightened electrical instability of the heart may differ between exercise and mental stress and not solely depend on the beta-adrenergic system activation. There were no statistically significant associations between markers of autonomic dysregulation of the heart (HRR and HF of HRV) and TWA. These may imply that markers of autonomic dysregulation of the heart do not measure the same physiological phenomena, increased arrhythmic vulnerability, in patients as TWA. Patients with increased myocardial vulnerability (e.g. QTVI) and myocardial substrate abnormalities (e.g. EF) at rest had high levels of MMA-TWA during exercise and mental stress, suggesting that both, resting QTVI and resting EF, reflect the same phenomenon as TWA – lability of repolarization processes in the heart. The moderate correlation of QTVI and EF with TWA implies that these indices are not duplicates and may have their unique contribution in the SCD risk stratification of patients. This further highlights the importance of composite vulnerability index assessment in patients who may be at risk for future cardiac arrhythmias. Additionally, the relationships of the markers of the arrhythmic vulnerability to TWA did not differ in their magnitude and directions between exercise and mental stress challenges, despite the fact that patients had higher levels of TWA and HR during exercise in comparison to mental stress. This may emphasize the different central and autonomic pathways contributing to their respective, adverse impacts on the cardiovascular systems of vulnerable patients.

## Tables

Table 1

| Medical History and Health Habits (n = 43) | |
|---|---|
| Characteristics | Count (%) |
| Current smoker | 7 (16.3%) |
| Beta Blockers (during study) | 30 (69.8%) |
| Nitrates (during study) | 11 (25.6%) |
| History of myocardial infarction | 37 (86%) |
| History of congestive heart failure | 5 (3.4%) |
| History of cardimyopathy | 18 (42.9%) |
| History of LV hypertrophy | 3 (8.1%) |
| History of diabetes | 13 (30.2%) |
| History of hypertension | 29 (67.4%) |

Table 2

| | Exercise | | Mental stress | | |
|---|---|---|---|---|---|
| | Mean (SD) | | | | |
| | | n | | n | |
| MMA-TWA (μV) | 6.5 (2.33) | 33 | 5.4 (2.89) | 43 | * |
| HR at max TWA (bpm) | 104.2 (9.16) | 33 | 91.7 (13.99) | 43 | * |
| Max HR (bpm) | 113.0 (15.40) | 41 | 78.3 (14.09) | 42 | * |
| Resting HR (bpm) | 65.8 (9.40) | 42 | 66.7 (10.55) | 33 | |
| HRR at 2.5 min (bpm) | 37.1 (14.07) | 40 | 10.3 (6.41) | 42 | * |
| Resting QTVI | -1.1 (0.05) | 37 | -1.1 (0.05) | 37 | |
| QTVI | -0.86 (0.63) | 38 | -0.71 (0.60) | 36 | |
| HF (log) | 1.2 (0.71) | 31 | 2.0 (0.68) | 36 | * |
| | Count (%) | | | | |
| | | n | | n | |
| MMA-TWA categorical | 25 (75.8%) | 33 | 21 (48.8%) | 43 | |
| MTWA | 27 (65.9%) | 41 | - | - | |
| Ischemia | 13 (41.9%) | 31 | 11 (35.5%) | 31 | |
| Resting EF | 31 (81.6%) | 38 | 31 (81.6%) | 38 | |

Table 3
Agreement between exercise and mental stress MMA-TWA (n = 33)

| | | Exercise MMA-TWA | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Mental Stress MMA-TWA | Positive | 15 (45.5%) | 2 (6.0%) | 17 (51.5%) |
| | Negative | 10 (30.3%) | 6 (18.2%) | 16 (48.5%) |
| | Total | 25 (75.8%) | 8 (24.2%) | 33 |

Note: Numbers within parentheses indicate percent of 33 patients – those who had data on both mental stress and exercise categorical MMA-TWA. Fisher's exact test: p = .118, Kappa Coefficient = .26, p = .085, showed that there was no association between exercise and mental stress MMA-TWA. In particular, 60% of patients who had positive TWA result during exercise also had positive TWA result during mental stress; and 75% of patients who were indentified as negative on TWA during exercise also had negative TWA result during mental stress.

Table 4

Agreement between exercise and mental stress myocardial ischemia (n = 31)

| | | Exercise Ischemia | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| Mental Stress Ischemia | Positive | 8 (28.8%) | 3 (9.7%) | 11 (35.5%) |
| | Negative | 5 (16.1%) | 15 (48.4%) | 20 (64.5%) |
| | Total | 13 (41.9%) | 18 (58.1%) | 31 |

Note: Numbers within parentheses indicate percent of 31 patients – those who had data on both mental stress and exercise ischemia. Results of Fisher's exact test ($p = .021$) and also Kappa Coefficient (Coefficient = $= 0.46$, $p = .01$) showed that there was an association between exercise and mental stress ischemia. In particular, patients who had ischemia during mental stress were more likely to have ischemia during exercise; and, likewise, patients who did not have ischemia during mental stress were less likely to have ischemia during exercise.

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Table 5a

Results of Repeated Analysis of Covariance: Comparing MMA-TWA between exercise and mental stress while controlling for Heart Rate during each challenge

| Independent Variable | Numerator df | Denominator df | F | p |
|---|---|---|---|---|
| Type of Challenge (Exercise versus Mental Stress) | 1 | 54.6 | 11.8 | 0.03 |
| HR (covariate) | 1 | 68.8 | 1.1 | 0.29 |

Note. The dependent variable was MMA-TWA.

Table 5b

Results of Repeated Analysis of Covariance: Comparing HRR between exercise and mental stress while controlling for Maximum Heart Rate during each challenge

| Independent Variable | Numerator df | Denominator df | F | p |
|---|---|---|---|---|
| Type of Challenge (Exercise versus Mental Stress) | 1 | 73 | 32.2 | <0.001 |
| Maximum HR (covariate) | 1 | 54.7 | 34.7 | <0.001 |

Note. The dependent variable was Heart Rate Recovery (HRR).

Table 6a

Bivariate Relationships between Non-invasive Markers and MMA-TWA during Exercise

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1. MMA-TWA | | | | | | | | | |
| 2. Resting QTVI | | 0.52** | | | | | | | |
| | n | 30 | | | | | | | |
| 3. Resting EF | | **-0.38*** | **-0.23** | | | | | | |
| | n | 28 | 33 | | | | | | |
| 4. Ischemia | | **0.1** | **0.17** | **-0.04** | | | | | |
| | n | 24 | 25 | 29 | | | | | |
| 5. HRR at 2.5min | | 0.01 | -0.05 | **-0.01** | **-0.06** | | | | |
| | n | 33 | 34 | 35 | 30 | | | | |
| 6. logHF | | 0.00 | -0.16 | **0.17** | **-0.19** | -0.18 | | | |
| | n | 24 | 27 | 27 | 23 | 27 | | | |
| 7. Number of MI | | -0.19 | -1.17 | **-0.29** | **-0.07** | 0.11 | 0.03 | | |
| | n | 33 | 31 | 38 | 31 | 40 | 31 | | |
| 8. Beta Blockers | | **-0.18** | **0.07** | **0.3** | **-0.06** | **-0.04** | **-0.17** | **0.06** | |
| | n | 33 | 36 | 37 | 30 | 40 | 31 | 42 | |

Note. Spearman's Rank-Order Correlation Coefficients are in bold-face. Pearson Correlation Coefficients are in normal typeface. *p < .05; ** p < .01.

Table 6b

Bivariate Relationships between Non-invasive Markers and MMA-TWA during Mental stress

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 1. MMA-TWA | | | | | | | | | |
| 2. Resting QTVI | | 0.49** | | | | | | | |
| | n | 37 | | | | | | | |
| 3. Resting EF | | **-0.43**** | **-0.23** | | | | | | |
| | n | 38 | 33 | | | | | | |
| 4. Ischemia | | **0.05** | **0.10** | **0.02** | | | | | |
| | n | 31 | 25 | 29 | | | | | |
| 5. HRR at 2.5min | | -0.06 | -0.05 | **0.11** | **0.16** | | | | |
| | n | 36 | 34 | 37 | 30 | | | | |
| 6. logHF | | -0.06 | -0.12 | **-0.27** | **0.03** | 0.06 | | | |
| | n | 36 | 34 | 33 | 25 | 35 | | | |
| 7. Number of MI | | -0.24 | -1.17 | **-0.29** | **-0.07** | -0.17 | -0.02 | | |
| | n | 43 | 37 | 38 | 31 | 42 | 36 | | |
| 8. Beta Blockers | | **-0.23** | **0.07** | **0.30** | **-0.06** | **0.26** | **-0.27** | **0.06** | |
| | n | 42 | 36 | 37 | 30 | 42 | 35 | 42 | |

Note. Spearman's Rank-Order Correlation Coefficients are in bold-face. Pearson Correlation Coefficients are in normal typeface. *p < .05; ** p < .01.

Table 7a

Summary of Hierarchical Regression Analysis of MMA-TWA during Exercise

| Variable | β | t | p | Part correlation coefficient |
|---|---|---|---|---|
| Block 1 | | | | |
| Myocardial Infarction | -.12 | -1.1 | .28 | -.22 |
| Beta Blockers | -.23 | -.59 | .56 | -.12 |
| Block 1 Adjusted $R^2$=.001, p=.39 | | | | |
| Block 2 | | | | |
| Myocardial Infarction | -.06 | -.35 | .73 | -.19 |
| Beta Blockers | -.20 | -1.23 | .23 | -.05 |
| Resting QTVI | .56 | 3.49 | .002 | .53 |
| Ejection Fraction | -.28 | -1.7 | .10 | -.26 |
| Block 2 Adjusted $R^2$= .43, p=.003; $\Delta R$=.44 | | | | |

Table 7b

Summary of Hierarchical Regression Analysis of MMA-TWA during Mental Stress

| Variable | β | t | p | Part correlation coefficient |
|---|---|---|---|---|
| Block 1 | | | | |
| Myocardial Infarction | -.27 | -1.5 | .13 | -.29 |
| Beta Blockers | -.23 | -1.3 | .19 | -.25 |
| Block 1 Adjusted $R^2$ = .08, p = .12 | | | | |
| Block 2 | | | | |
| Myocardial Infarction | -.34 | -2.1 | .05 | -.29 |
| Beta Blockers | -.12 | -.77 | .45 | -.25 |
| Resting QTVI | .29 | 1.8 | .08 | .42 |
| Ejection Fraction | -.37 | -2.2 | .04 | -.36 |
| Block 2 Adjusted $R^2$ = .29, p = .01; $\Delta R$ = .25 | | | | |

Table 8a

Regression Analysis of MMA-TWA during Mental Stress:
The Collective Effect of Non-Invasive Markers

| Variable | β | t | p | part |
|---|---|---|---|---|
| Resting QTVI | .66 | 2.3 | .04 | .52 |
| Ejection Fraction | -.37 | -1.5 | .16 | -.34 |
| Prevalence of Ischemia | -.13 | -.40 | .70 | -.09 |
| High Frequency | -.14 | -.53 | .61 | -.12 |
| Heart Rate Recovery | .04 | .15 | .88 | .03 |

Note. Full Model Adjusted $R^2$ = .33, p = .13.

Table 8b

Summary of Regression Analysis of MMA-TWA during Exercise:
The Collective Effect of Non-Invasive Markers

| Variable | β | t | p | part |
|---|---|---|---|---|
| Resting QTVI | .31 | 1.6 | .13 | .29 |
| Ejection Fraction | -.41 | -2.2 | .05 | -.39 |
| Prevalence of Ischemia | -.27 | 1.5 | .17 | .26 |
| High Frequency | -.19 | -1.0 | .32 | -.19 |
| Heart Rate Recovery | .29 | -1.6 | .14 | -.28 |

Note. Full Model Adjusted $R^2$ = .36, p = .03.

Table 9
Agreement between MMA-TWA and MTWA (n = 32)

| | | MTWA (Cambridge Heart Method) | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| MMA-TWA | Positive | 19 (59.4%) | 5 (15.6%) | 24 (75%) |
| | Negative | 3 (9.4%) | 5 (15.6%) | 8 (25%) |
| | Total | 22 (68.8%) | 10 (31.2%) | 32 |

Note: Numbers within parentheses indicate percent of 32 patients – those who had data on both MMA-TWA and MTWA during exercise challenge.

Table 10
Agreement between MTWA and EF (n = 36)

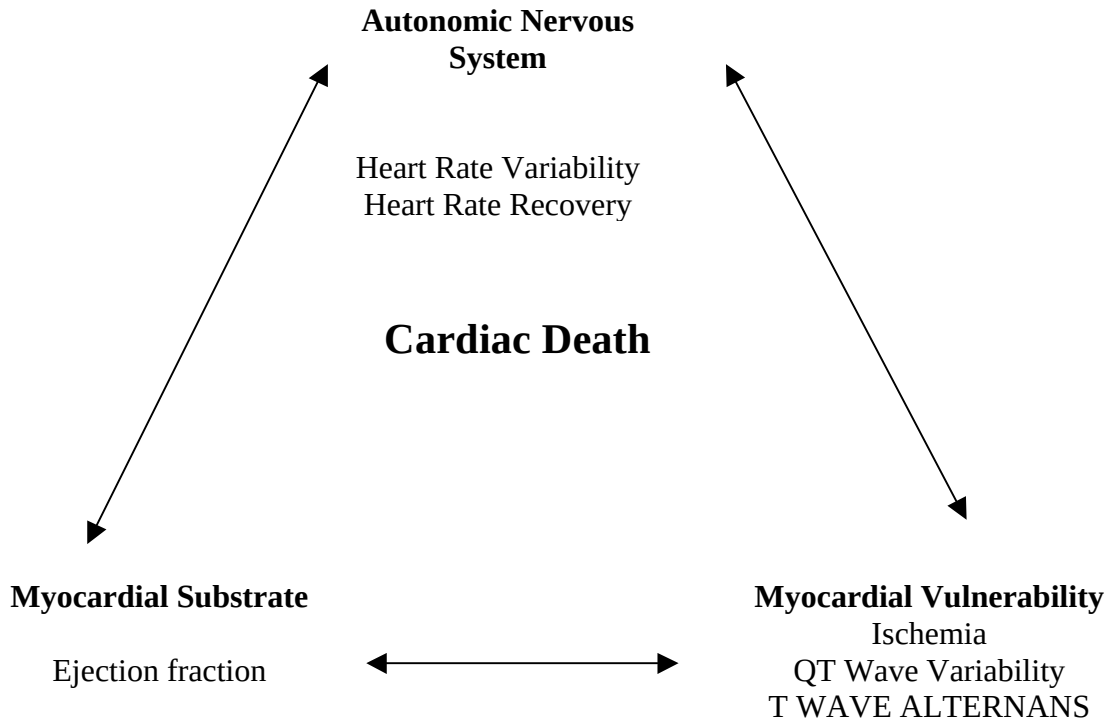
| | | MTWA (Cambridge Heart Method) | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| EF | Low (<40) | 19 (52.8%) | 10 (27.8%) | 29 (80.6%) |
| | High (>40) | 4 (11.1%) | 3 (8.3%) | 7 (19.4%) |
| | Total | 23 (63.9%) | 13 (36.1%) | 36 |

Note: Numbers within parentheses indicate percent of 36 patients – those who had data on both EF and MTWA during exercise challenge.
Results showed that sensitivity was 82.6%; that is, patients with low EF also had positive MTWA results on 82.6% of the cases identified as positive by the MTWA testing during exercise. Specificity was 23.1%; that is patients with high EF also had negative MTWA results on 23.1% of the cases identified as negative by the MTWA testing during exercise.

**Figures**

Figure 1.

Lethal Triad




Adapted from Zareba & Moss (2003)

Figure 2.
Conduction system of the heart

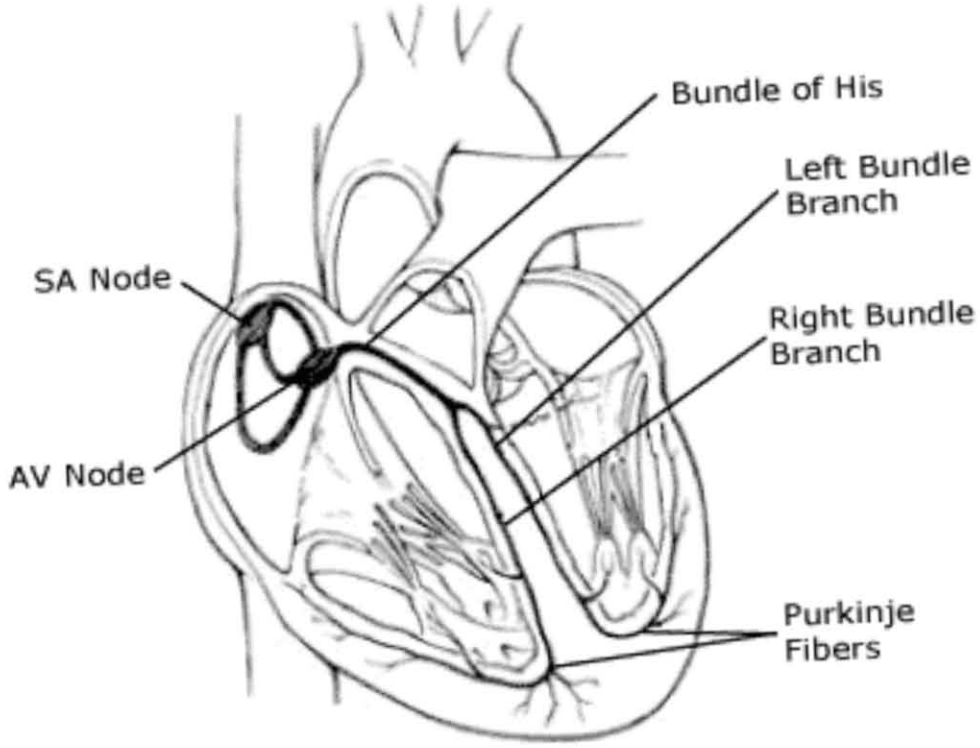
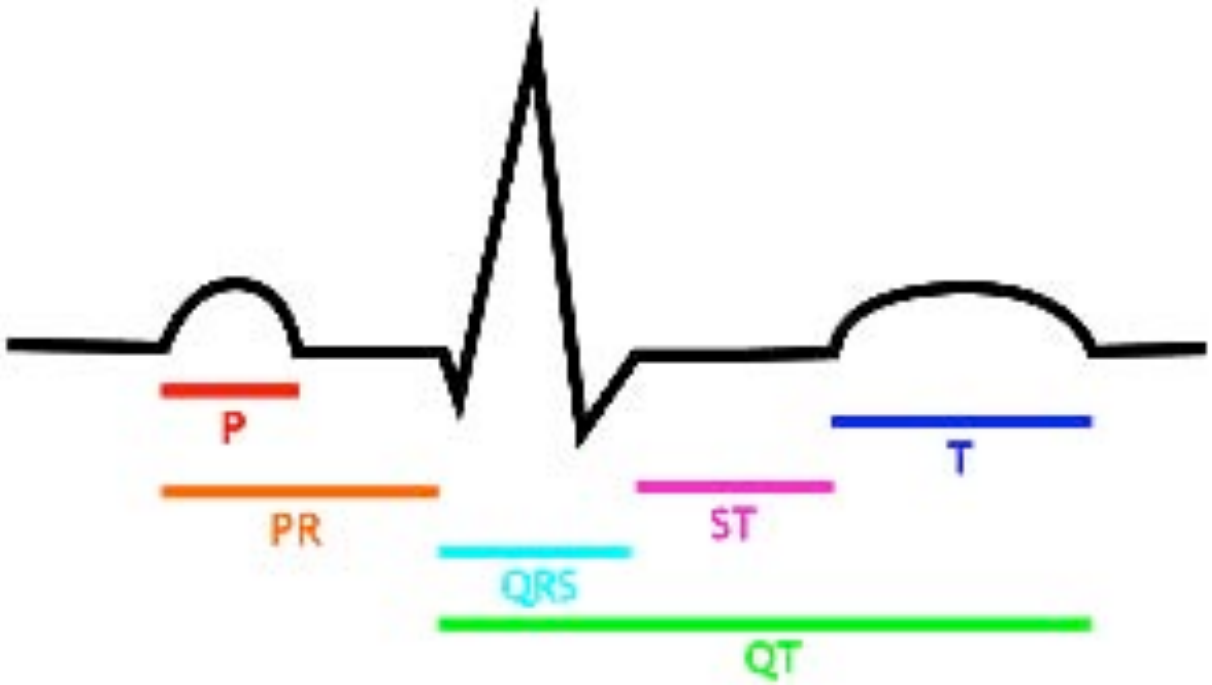


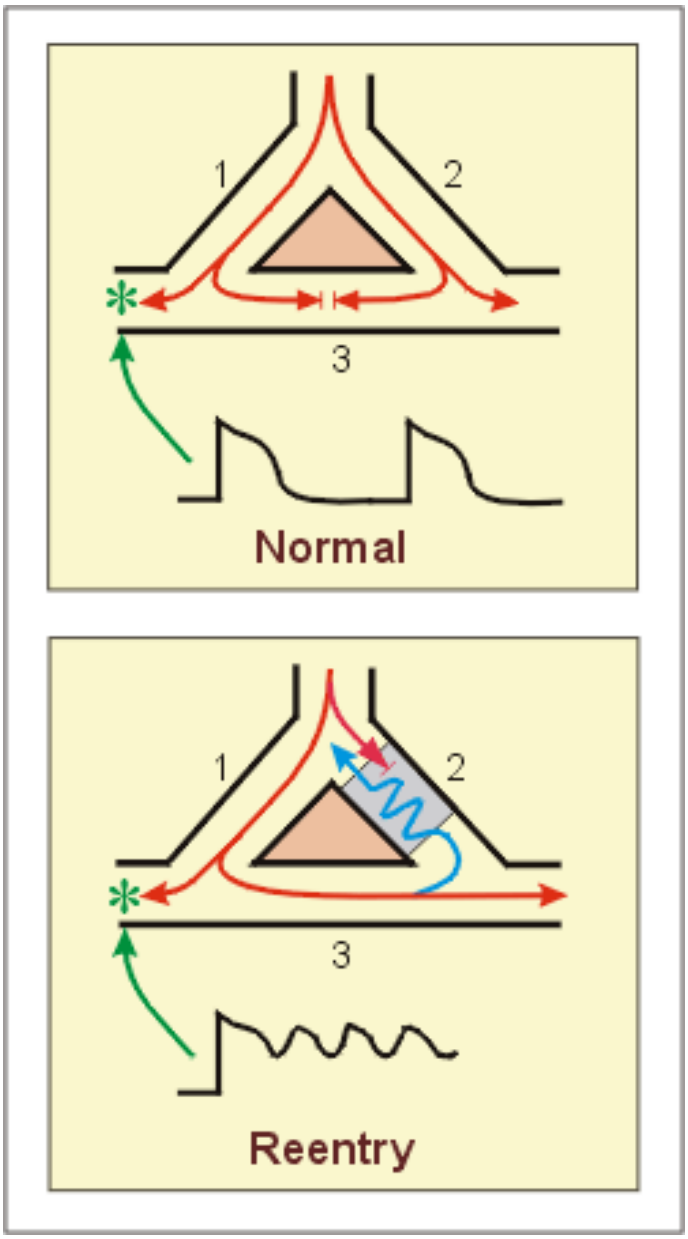
Figure 3.
Electrocardiogram




The ECG complex. P=P wave, PR=PR interval, QRS=QRS complex, QT=QT interval, ST=ST segment, T=T wave

*Note:* retrived from http://vyanayoga.com/in-a-heartbeat.php

Figure 4.
Illustration of re-entry phenomenon

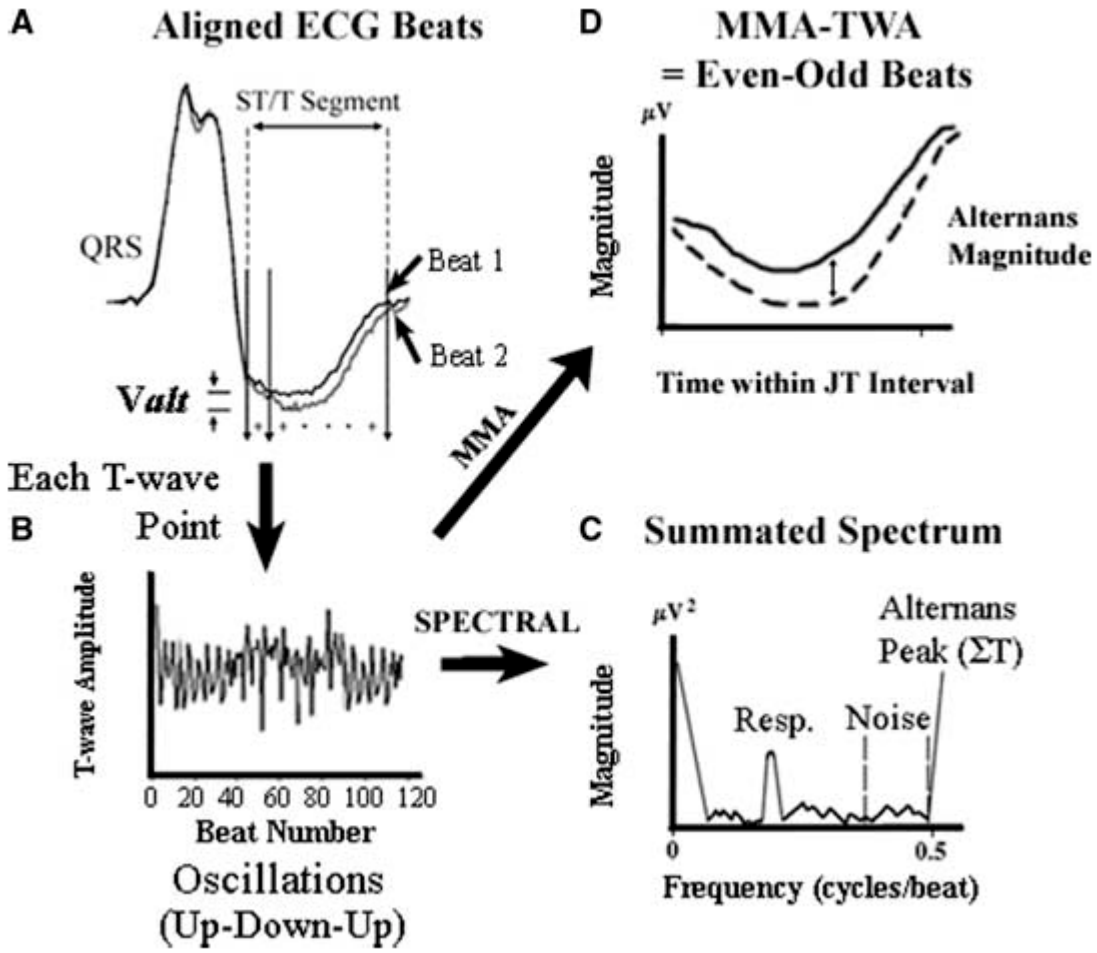



Adapted from Klaunde et al. 2007

The top figure illustrates normal propagation of the impulse, moving outward and stopping (3), through the heart.

The figure on the bottom illustrates the self-regenerating conduction of impulses, independent of the SA nodal rate. The unidirectional block (2) impairs conduction in one direction (2) and allows conduction of the impulse in opposite direction (3). Allowing impulses travel in a circle within the heart (3).

Figure 5
T-wave alternans computation for the Microvolt T-wave alternans (MTWA) and Modified Moving Average (MMA) methods




Adapted from Cox et al., 2006

TWA is Computed as follows: (A) Beats are aligned by QRS complexes. At each successive time-point in the aligned T-waves (arrows), (B) Observed beat-to-beat oscillations in the T-wave reflect alternans at each time-point; (C) The MTWA method uses spectral analysis that applies the fast Fourier transformation to yield a power spectrum in which alternans is the peak at the frequency of half the heart rate (0.5 cycles/beat). (D) The alternative method called MMA analysis uses a nonlinear filter to quantify the maximum difference between the means of 'even' versus 'odd' beats in an alternating sequence.

Figure 6
Example of TWA-MMA summary table

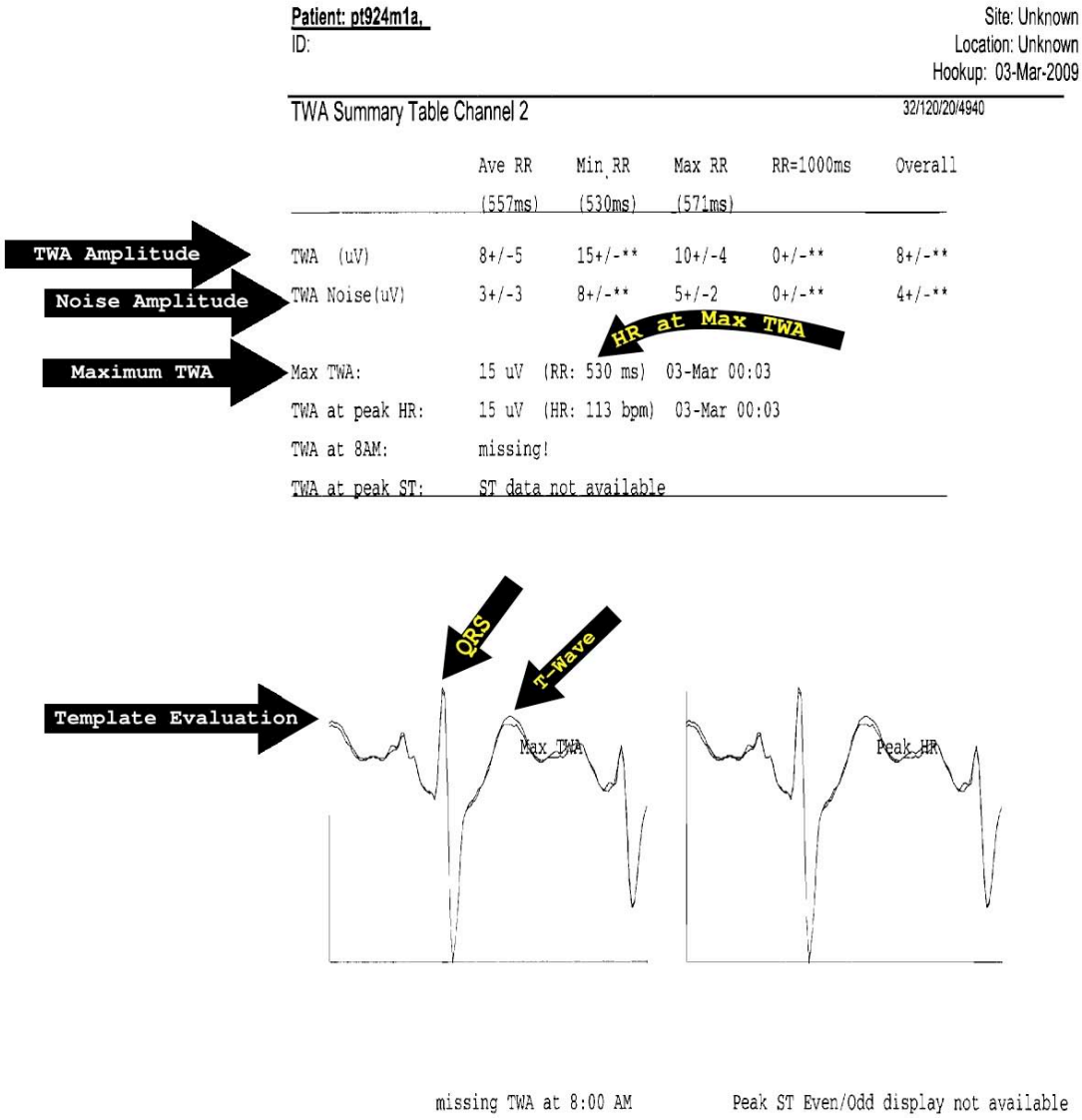
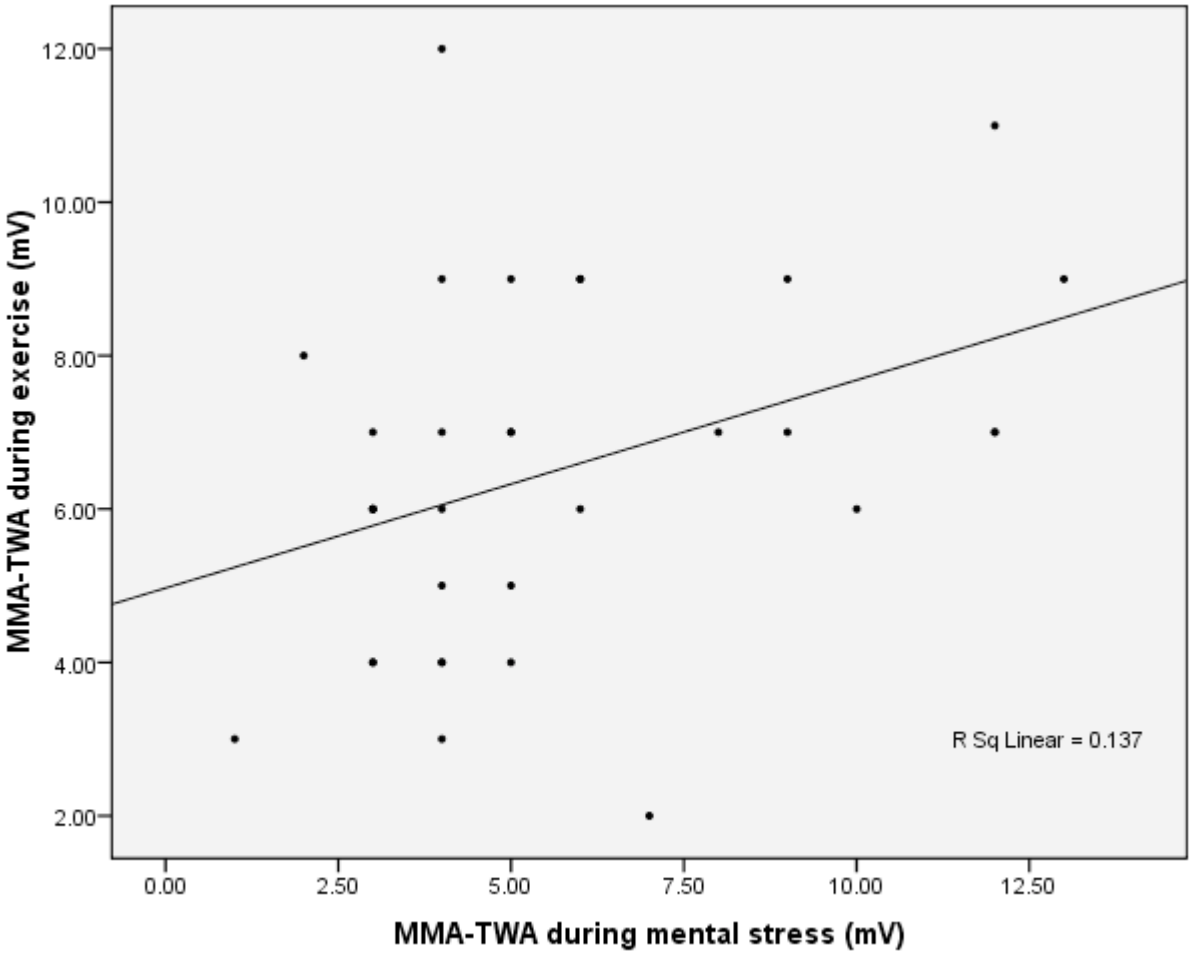
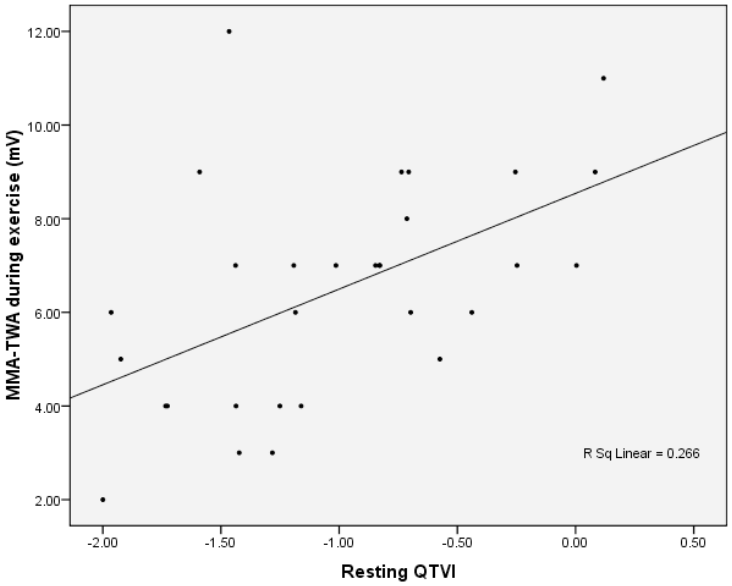


Figure 7
Association between Exercise MMA-TWA and Mental Stress MMA-TWA

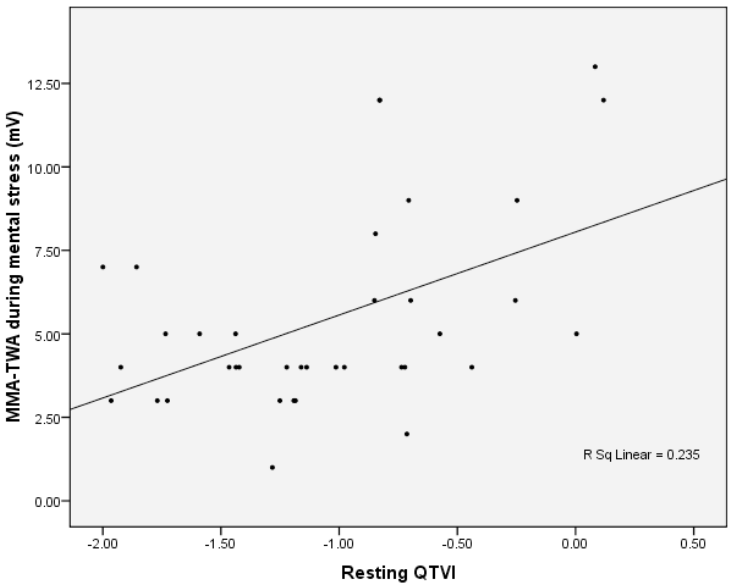



Note. $r_p = 0.37$, $p = 0.034$

Figure 8
Associations of resting QTVI with TWA-MMA during exercise and mental stress

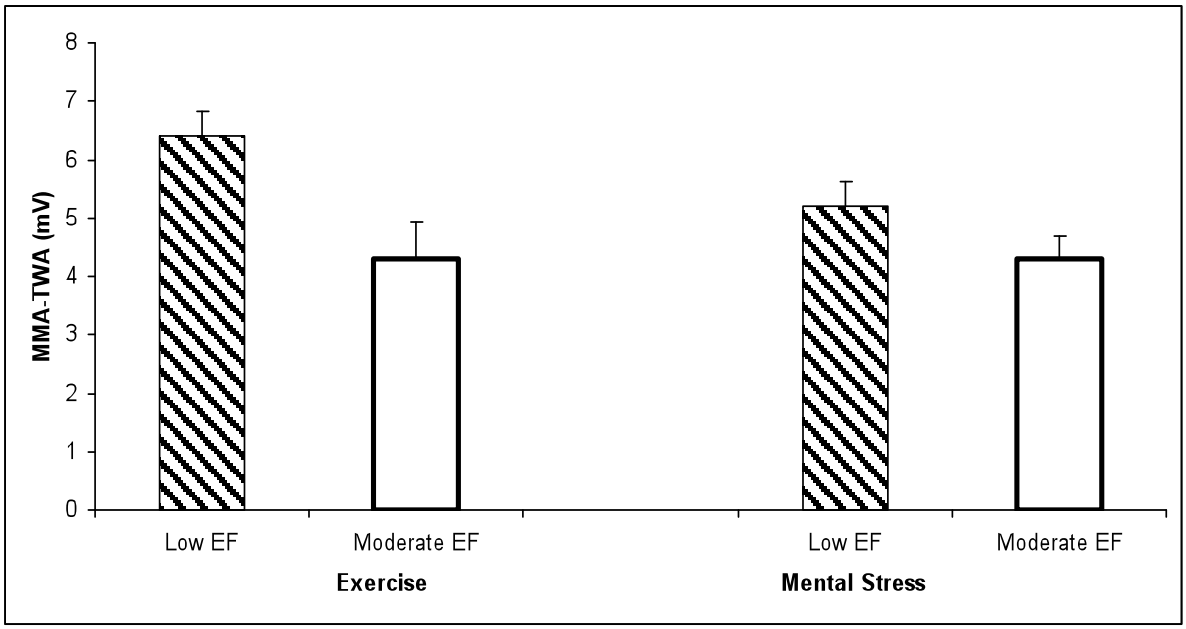



Note: $r_p = 0.52$, $p = 0.004$



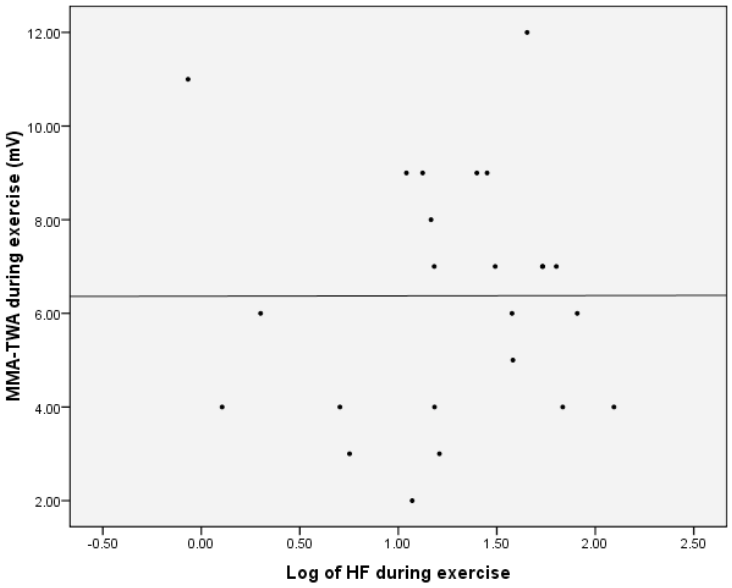

Note: $r_p = 0.48$, $p = 0.002$

Figure 9
Associations of ejection fraction with TWA-MMA during exercise and mental stress

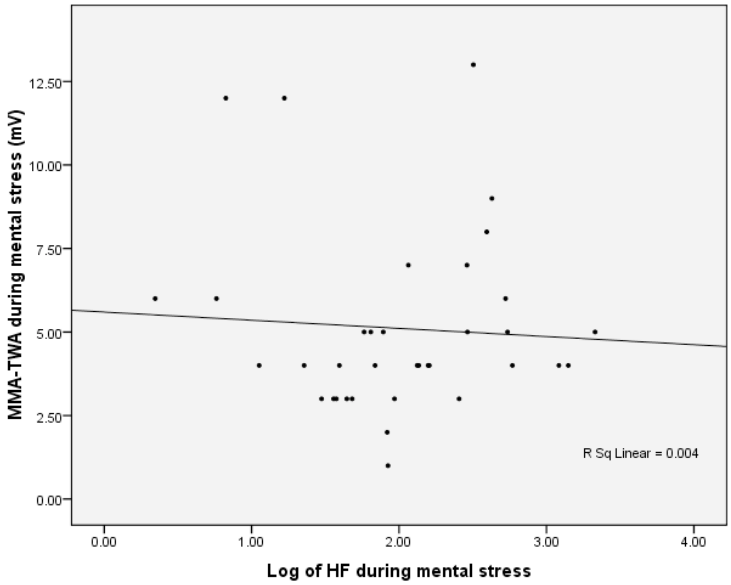



Note: Exercise $r_{sr}$= -0.38, p = 0.04; Mental Stress $r_{sr}$= -0.43, p = 0.007
Low EF ≤40%, Moderate EF>40%

Figure 10
Associations of HF with TWA-MMA during exercise and mental stress

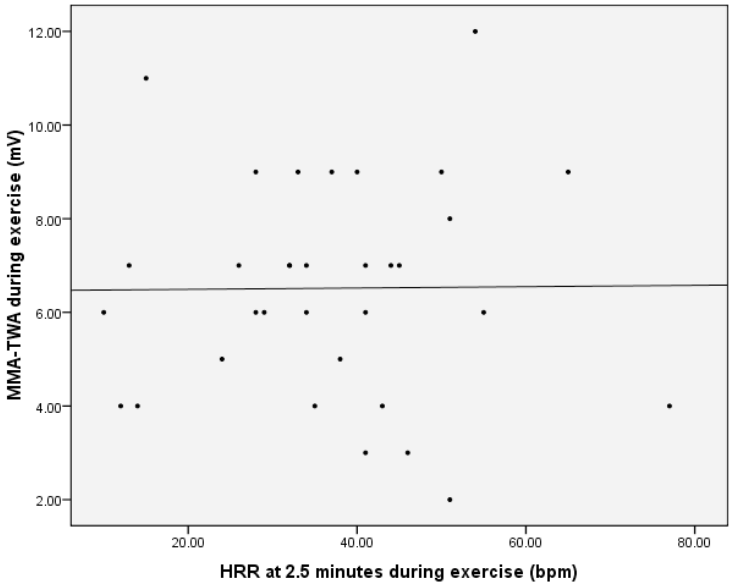



Note: $r_p = 0.00$, $p>0.5$

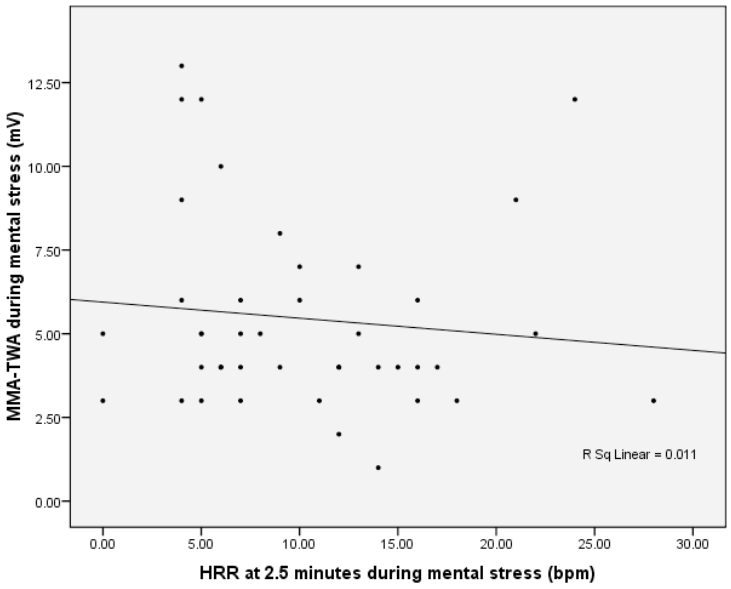



Note: $r_p = -0.06$, $p >0.5$

Figure 11
Associations of HRR with TWA-MMA during exercise and mental stress



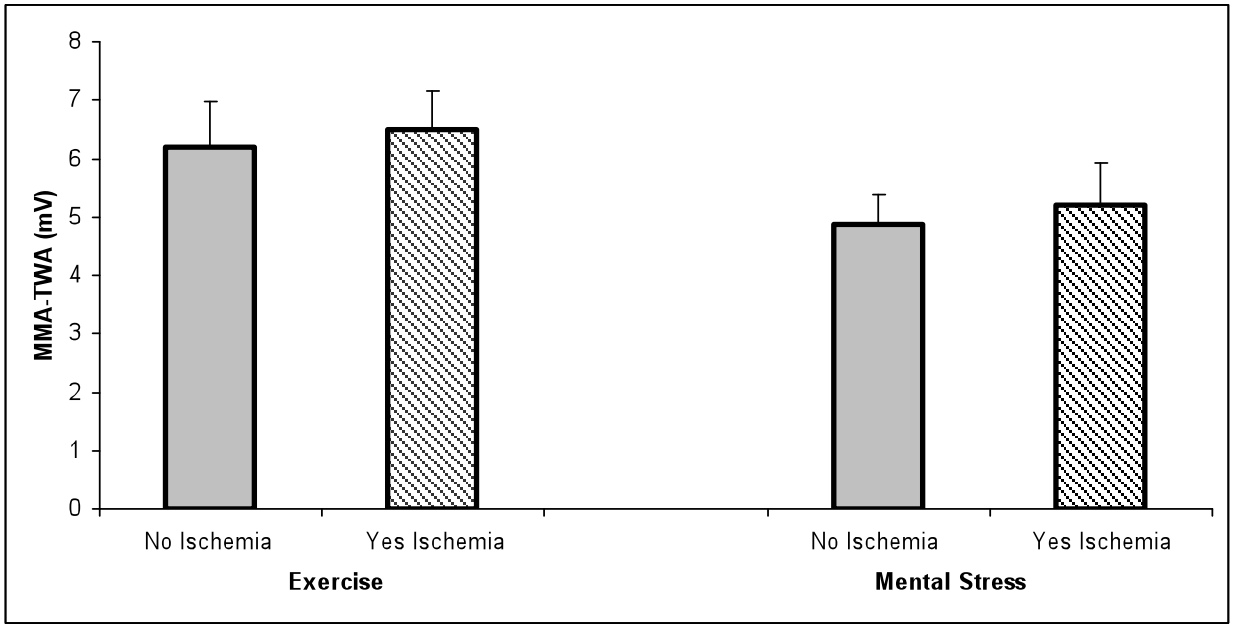

Note: $r_p = 0.01$, $p > 0.5$




Note: $r_p = -0.06$, $p > 0.5$

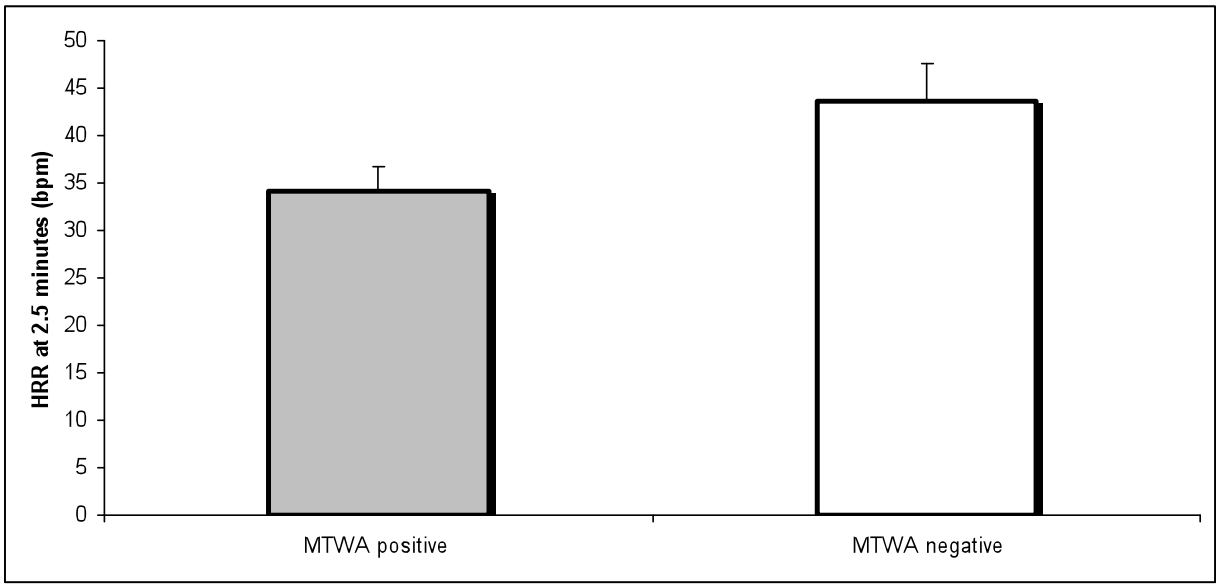
Figure 12

Associations of myocardial ischemia with TWA-MMA during exercise and mental stress

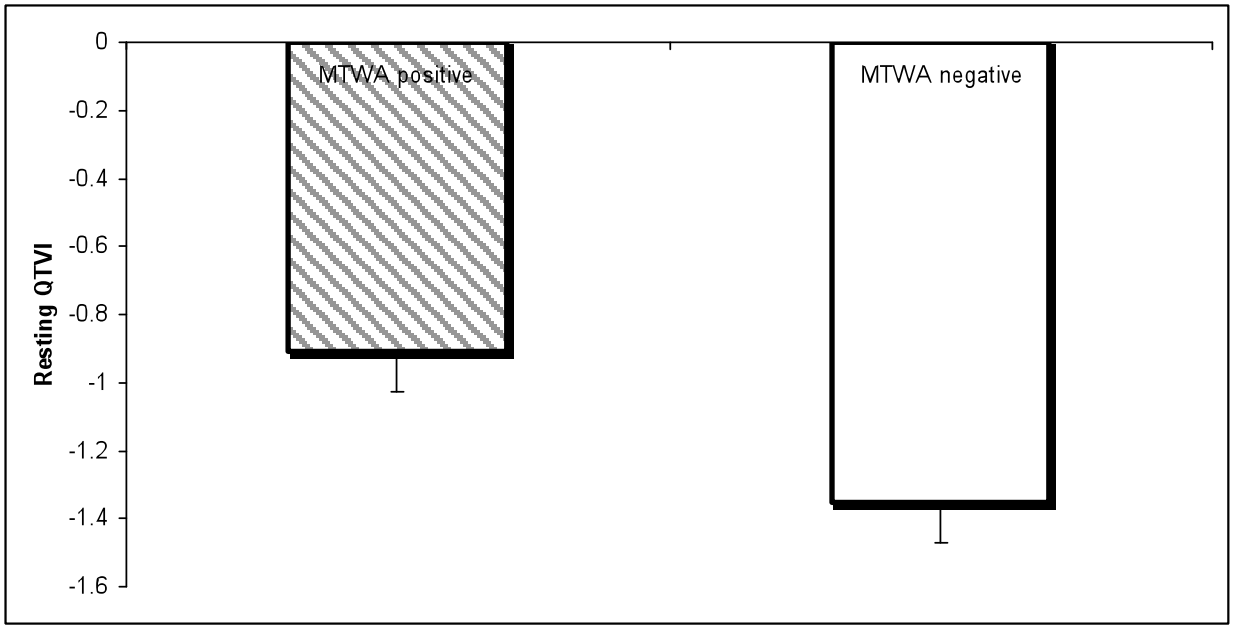



Note: Exercise $r_{sr}$= 0.1, p >.05
Mental Stress $r_{sr}$= 0.05, p >.05

Figure 13
Associations of MTWA with HRR at 2.5 minutes and resting QTVI during exercise




Note: Exercise $r_{sr}$= -0.33, p =0.04




Note: Exercise $r_{sr}$= 0.39, p =0.02

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